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THE EFFECTS OF THE SULFONYLUREAS AND RELATED COMPOUNDS IN EXPERIMENTAL AND CLINICAL DIABETES

BY

RACHMIEL LEVINE (*Conference Chairman*), I. P. ACKERMAN, J. APELBAUM, J. ASHMORE, A. BÄNDER, S. B. BEASER, D. M. BERGENSTAL, B. R. BOSHELL, J. BOWKETT, A. E. BRAVERMAN, B. BRITTON, G. F. CAHILL, JR., R. CAMERINI-DÁVALOS, M. CAMMARN, M. M. CAMUS, A. R. COLWELL, JR., A. R. COLWELL, SR., J. A. COLWELL, J. W. CONN, J. W. CRAIG, H. DOLGER, T. DORFMÜLLER, N. W. DREY, W. R. DRUCKER, W. E. DULIN, G. G. DUNCAN, A. S. EARLE, H. ELRICK, W. ENDRES, J. ENZINGER, V. O. ERK, S. S. FAJANS, G. L. FISCHER, J. FOLEY, T. F. FRAWLEY, M. FURTHMÜLLER, D. D. GELLMAN, S. GITELSON, R. D. GITTLER, F. C. GOETZ, M. G. GOLDNER, H. O. HAAR, L. F. HALLMAN, J. D. HAMILTON, A. R. HENNES, B. A. HOUSSAY, R. D. JOHNSON, R. L. JOHNSTON, M. KARASEK, R. M. KARK, J. J. KRAKE, S. S. LAZARUS, C. T. LEE, A. LOUBATIÈRES, L. H. LOUIS, H. A. LUBS, T. H. MCGAVACK, M. S. MACKENZIE, A. MARBLE, D. B. MARTIN, M. MILLER, W. L. MILLER, JR., I. A. MIRSKY, J. A. MOORHOUSE, J. C. PENHOS, G. PERISUTTI, R. PURNELL, L. RECANT, L. M. REINEKE, A. E. RENOLD, H. T. RICKETTS, H. SCHMID, J. A. SCHRICKER, W. SEEGERS, S. SEGAL, I. SEIDLER, S. SHERRY, G. STÖTTER, S. J. N. SUGAR, N. TEODOSIO, G. W. THORN, E. URGOITI, M. J. VANDER BROOK, M. VAUGHAN, B. W. VOLK, B. L. WAJCHENBERG, S. WEISENFELD, A. N. WICK, H. L. WILDBERGER, H. WOODWARD, JR., G. A. WRENSHALL, J. K. YOUNG, J. L. ZEFFREN.

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Conference Chairman and Consulting Editor

RACHMIEL LEVINE

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INTRODUCTION

By Rachmiel Levine
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The present conference is a consequence of deliberate and well-planned research based upon a series of chance observations in a field far removed from diabetes. But for M. Janbon's fortuitous observation that one of the newer sulfonamides of 1942 produced a disorder very similar to hypoglycemia, Auguste Loubatières very probably would not have devoted the next fifteen years to an effort to ascertain the nature of these symptoms and to analyze the mechanism which produced them. The sulfonylureas were also originally designed as more soluble sulfa drugs with prolonged action, and their effects in diabetes were consequences of unexpected activity.

Loubatières was prepared to look for the mechanism of action of these drugs by reason of his long experience in experimental diabetes stemming directly to Hédon and through him to Minkowski.

The brilliant and extensive work on the relation of endocrines to the diabetic state by Houssay and his school in Argentina has never been dimmed by the political vicissitudes which he and his colleagues suffered for many years. Also in this latest field of research in diabetes, his authoritative voice speaks from deep experience.

It is to the credit of all workers in the field, that, within a relatively short space of time, we have learned so much about the sulfonylureas. Within limits, standards of tolerance, dosage, and effectiveness have been established. Indications for possible clinical use are in general agreed upon. The mechanisms of action have become clearer, although not yet fully understood. We have come to the important recognition that such drugs, convenient and valuable as they may prove to be, do not relieve the physician or the patient from the obligation to exercise strict dietary control nor do they free the diabetic from learning to use insulin in an intelligent manner.

Transcending any of the clinical considerations brought forth by these drugs, their effect has already been forcefully felt in the stimulus they have given to research into the intimate aspects of insulin production, release, and secretion; into the highways and byways of insulin metabolism; and into the vexing problem of the etiological factors concerned in the production and appearance of diabetes mellitus in man.

THE HYPOGLYCEMIC SULFONAMIDES: HISTORY AND DEVELOPMENT OF THE PROBLEM FROM 1942 TO 1955

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Clinical Observations and First Hypothesis (1942)

The first clinical and experimental observations that formed the background for the utilization of certain sulfonamide derivatives in the treatment of diabetes were made during the first half of 1942. At that time M. Janbon and his co-workers of the Infectious Disease Clinic at the Medical School of Montpellier were investigating the therapeutic effect of the isopropylthiodiazole derivative of sulfanilamide (2254 RP) in typhoid fever. They found that this drug produced in some patients, especially those who were undernourished, symptoms and signs resembling hypoglycemia. Some patients had grave neurological disturbances, and died. The chemical data revealed low values for blood sugar. The intravenous injection of glucose tended to alleviate the symptoms in some individuals, but in others it was not helpful. Janbon presented this problem to us and asked for our advice.

Some months earlier we had completed an experimental study with Hédon and Heymann[1, 2] of the irreversible neurological lesions produced in the dog by large doses of protamine zinc insulin. It occurred to me that there might be a parallelism between the effects of 2254 RP and of protamine zinc insulin. This was, of course, a tentative idea, and it was necessary to submit it to experimental test.

Physiological Analysis of the Mechanism of Action of 2254 RP (1942 to 1946)

This study began in June of 1942. I first determined that the addition of the drug to blood did not affect the determination of sugar by the method of Hagedorn and Jensen, so that the values obtained were not due to artifacts. Next, I determined that the oral administration of 2254 RP (0.25 gm. per kg.) to a normal fasting dog resulted in a progressive hypoglycemia, with blood sugar as low as 50 mg. per cent. In these acute experiments the degree of hypoglycemia was a function of the blood sulfonamide level. I observed that 24 hours after the administration of the drug the blood sugar still had not attained its initial value. In a completely depancreatized dog the administration of 2254 RP by mouth or by subcutaneous or intramuscular injection had no effect on the level of blood sugar. I therefore adopted the hypothesis that the pancreas was necessary for the action of the drug and that this substance was pancreatotropic, acting upon the endocrine system of the islets of Langerhans and stimulating the processes involved in the secretion of insulin.[3, 4, 5, 6] I informed Janbon of these experimental results and of the hypothesis. On July 3, 1942, Janbon and his co-workers presented to the Society of Medical and Biological Sciences of Montpellier their clinical experiences and mentioned the above initial experimental results.[7, 8, 9, 10] Later I reported to Janbon that I had produced convulsive seizures in dogs by administering the drug for many successive days. Initially, these hypoglycemic symptoms could be reversed by glucose injection, but they became irreversible when glucose therapy was instituted too late after the beginning of hypoglycemia. These phenomena were exactly like those which follow the injection of prolonged and excessive use of protamine zinc insulin.[1, 2] My initial hypothesis seemed confirmed and, in October of 1942, Janbon and his co-workers and my colleagues and I presented our results during the 43rd Congress of the French-Speaking Neurologists.[3, 11]

Despite the many difficulties occasioned by the war period, between 1942 and 1946 I pursued the study of the mechanism of action of the hypoglycemic sulfonamides and demonstrated a number of facts that recently have assumed some importance. These are described in four basic publications.[4, 5, 6, 12] I shall try to summarize these facts briefly:

(1) The hypoglycemic action of 2254 RP cannot be obtained in the completely depancreatized animal, but it can be shown to occur if the animal retains as little as one sixth of its pancreas.

(2) The lowering of the blood sugar is not due to a stimulation of nerve centers transmitted to the pancreas by the vagi, since vagotomy or the administration of atropine does not interfere with the hypoglycemia.

(3) The hypoglycemia is due to a direct stimulation of the insulin-secreting cells. The slow injection of a small dose of the sodium salt of 2254 RP into the artery supplying the uncinate process of the pancreas produces definite lowering of the blood sugar. When one injects a similar amount of the sodium salt into the glandular end of the main pancreatic duct of the dog one obtains, after a brief hyperglycemia, a slow but profound lowering of the blood sugar (30 mg. per cent). A control NaOH solution produces variations in blood sugar that are not significant.

(4) It is possible to demonstrate insulin liberation by making a vascular anastomosis, according to the method of Zunz and La Barre, between the pancreaticoduodenal vein of a donor dog (which receives the drug) and the jugular vein of a recipient dog previously rendered diabetic by alloxan.

(5) At this stage I considered that "the concentration of the sulfonamide in contact with the islet cells was the factor responsible for the liberation of insulin" and that the drug was "an agent exciting insulin secretion."[6]

If this were so, one should be able to demonstrate that under certain experimental conditions 2254 RP would produce metabolic changes similar to those of insulin. I found that this substance permitted the accumulation of significant amounts of glycogen in the liver of a dog that had been fasted for four days in order to sensitize it to the effects of insulin. The sulfonamide did not elevate the R. Q. of a totally depancreatized dog during glucose absorption. This showed that the drug did not act directly to increase carbohydrate combustion. The R. Q. was elevated in an incompletely depancreatized dog, but this could be explained by the liberation of endogenous insulin.

I tested other hypotheses that could explain the hypoglycemic action of this sulfonamide. In 1946[5, 6] I showed that 2254 RP did not inhibit the diabetogenic effect of anterior pituitary extract. Other experiments showed that *in vitro* the drug did not affect glycolysis in the heparinized blood of the dog.

In 1946 it occurred to me to test whether 2254 RP had a trophic action on the islets of Langerhans. In rabbits given 0.5 gm. per kg. of the drug by mouth every day for three weeks the pancreas showed hyperplasia of the islets. The cells themselves were increased in size and their nuclei also were larger than normal. They appeared active, without any degenerative manifestations. It was also observed that the other organs of the animal, in particular the liver, showed no histological abnormalities.

At that time we also reported[5, 13] attempts to treat alloxan diabetes of the rabbit by the daily administration of 2254 RP. In rabbits in which the blood sugar was reduced by fasting and in which biopsy showed that some islets were preserved, the oral administration of the drug reduced the blood sugar and the glycosuria consequent to food, and the weight became stable. When the drug was discontinued the diabetes became progressively worse. We attributed this antidiabetic action to stimulation of the remaining islets.[5, 13] In a similar manner we interpreted[14] the very feeble hypoglycemia obtained by injection of the sulfonamide into an alloxanized dog. We had also observed that a certain number of alloxan diabetic animals treated with 2254 RP were "cured" of their diabetes. Although new formation of islets could explain such a phenomenon, we speculated on other possible mechanisms. Since 1946 investigation of these possibilities has been the objective of our work.[15, 16, 17]

Following our publications of 1942 to 1946, a few authors became interested in the mechanism of action of the hypoglycemic sulfonamides. Chen, Anderson, and Maze[18] utilized a substance closely related to 2254 RP—the cyclopropyl derivative—and found that it produced hypoglycemia in the normal rabbit and hyperglycemia in the severely alloxanized animal. They concluded that their substance stimulated insulin secretion, in conformity with our hypothesis.

La Barre and Reuse[19] confirmed the hypoglycemic action of 2254 RP in the dog and showed that this was more intense after adrenalectomy. They found, as we had, that hypoglycemia could be elicited in incompletely alloxanized animals, but not in those in which the diabetes was produced by large doses of alloxan. They concluded, in confirmation of our hypothesis, that the drug possesses the property of stimulating the endocrine function of the pancreas.

There were very few in 1946 who had any notion of the eventual interest in these hypoglycemic compounds. At that time I wrote: "One might think of establishing a test by giving 2254 RP to diabetics in order to determine their capacity for insulin secretion. It is possible to imagine diabetic states resulting purely from a weakening of the insulin secretion process."[5] In underlining the therapeutic interest of this drug and of related chemicals I also wrote: "It is logical to assume that such hypoglycemic agents could eventually be used in the treatment of certain forms of diabetes. One must suppose that in addition to diabetes that results from anatomical damage to the islets of Langerhans there may be another functional form, as a consequence of deficiency of insulin secretory mechanisms. In the latter case the islet cells might appear histologically normal, even though they have an abnormal excitation threshold, and would therefore liberate less insulin than is necessary to maintain a normal blood sugar level. It is in this type of diabetes that it would appear logical to utilize the sulfonamide compound with which we have worked."[5]

Pharmacodynamic Investigations: Relation Between Structure and Activity (1942 to 1946)

In addition to the physiological studies, pharmacological investigations were conducted between 1942 and 1946 with the aim of establishing relationships between structure and activity. Janbon predicted them in 1943.[20] They were accomplished by Bovet, Dubost, and Loubatières, using about 20 derivatives.

Bovet and Dubost[21] worked with the dog. Loubatières used dogs and rabbits[5, 6, 12, 13] and reached similar conclusions. These papers were to appear simultaneously, but because of the conditions during the eventful year of 1944, our note appeared a month later than that of Bovet. Our conclusions do not differ significantly from those of the latter. This study revealed that among close derivatives of the isopropyl compound some were frankly hypoglycemic, others exhibited a strong hyperglycemic action, and others had only slight activity.[5] The hyperglycemic sulfonamides probably acted in the same way as has been reported for sulfathiazole and sulfanilamide, which Goldner and Ricketts[22] found to be capable of diminishing glucose tolerance when given in large doses. In 1946 we drew the following conclusions about structure-activity relationships: "The sulfonamide compounds that depress the blood sugar seem to form a group of thiodiazole derivatives. Among them the tertiary butyl and isobutyl are the most active, then in order of decreasing activity the butyl, amyl, isoamyl, propyl, and isopropyl compounds. The ethyl and methyl derivatives possess no activity. The hexyl, heptyl, and amino thiodiazole compounds have insignificant activity."

In 1946 we drew attention[5, 6, 13] also to the significance of the side chain attached to the thiodiazole nucleus. We had found that alcohols corresponding to these hydrocarbon chains, particularly isopropyl, butyl, and amyl alcohols, themselves exert some hypoglycemic activity. We thought that the activity of these drugs that we had studied was the result of the action of the hydrocarbon chain, with the *p*-aminobenzene portion acting as a reinforcing agent to the rest of the molecule.[6]

Investigations Between 1947 and 1955

In 1951 and 1952, after we had studied the possible role of "endogenous alloxan or alloxanoid substances" in the pathogenesis of diabetes,[17, 23] we established the fact that 2254 RP seems to inhibit the formation of endogenous "alloxan" both *in vitro* and *in vivo*. In 1952 Davis[24] showed that the subcutaneous injection of the hydrochloride or sulfate salt of synthalin A (decamethylene diguanidine) into rabbits produced hydropic degeneration of the α cells of the islets of Langerhans. In 1954 and 1955 von Holt and his co-workers utilized 2254 RP, which they renamed IPTD, for a study of its effects on the α cells.[25, 26, 27] They confirmed the hypoglycemic action of this substance, and they also observed the phenomenon of attenuation of alloxan diabetes in the rabbit and its "cure" in certain cases, phenomena that we had already described. They found severe lesions of the α cells and, in many cases, their total disappearance. On the other hand the β cells were spared. In alloxanized animals "cured" by IPTD, von Holt explained the disappearance of diabetes by the complete destruction of the α cells. In such animals there were no functional islets at all.

The work of Creutzfeldt and Tecklenborg in 1955[28, 29] cast doubt on von Holt's theory. These authors stated that the hypoglycemia manifests itself before any alteration is seen in the α cells, and they could not confirm the disappearance of such cells. They stated that my hypothesis of stimulation of insulin secretion as a basis for the hypoglycemia seemed more likely.

Toward the end of 1954 and during the first months of 1955 we obtained evidence that the administration of 2254 RP in large doses produces degranulation of the α cells, but it was difficult to give a functional interpretation to such changes.[30, 31, 32] We have always maintained[33, 34, 35] that the primary and essential action of 2254 RP is stimulation of the secretion of insulin by the β cells. In particular, we had established that in recent or moderate alloxan diabetes, the activity of the sulfonamides is related to the number of β cells spared by the alloxan. We believe that the progressive improvement of diabetes elicited by 2254 RP in such animals depends upon the new formation of β cells from acinar or duct cells that we have shown takes place.[31, 32] It is true that we had considered the hypothesis that the improvement in diabetes from 2254 RP in alloxanized animals might be due to an action of the α cells. However, we have always considered such a mechanism, if it does operate, to be subsidiary to the essential actions on insulin secretion and new formation of β cells.

Whatever the intimate mechanism of such "cures or remissions" turns out to be, its location is definitely intrapancreatic. We demonstrated in 1955 that alloxan diabetic animals cured by 2254 RP became diabetic immediately after the total ablation of the pancreas.[32, 36]

On the 25th of September 1955, when we reported some of our work on the sulfonamides,[32] we were asked whether these had been tried in diabetic humans, what the results were, and whether we should distribute such compounds for clinical trial. At that time our clinical trials had begun, but we decided not to distribute such compounds until we were certain that they were reasonably nontoxic in humans.

On October 7, 1955, a group of German investigators published their first experimental and clinical results using the sulfonamide BZ 55.[37, 38, 39] The bibliography appended to these publications refers to one of our 1946 papers.[6] On November 14, 1955, we presented evidence that in the diabetic human 2254 RP has the same action as BZ 55.[40, 41, 42]

It appears therefore that 2254 RP can be considered as the first of a pharmacological and therapeutic group of agents of the sulfonamide variety that are hypoglycemic and antidiabetic. Recent work has served to confirm this general statement.[43]

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ACTION OF THE HYPOGLYCEMIC SULFONYL COMPOUNDS IN HYPOPHYSECTOMIZED, ADRENALECTOMIZED, AND DEPANCREATIZED ANIMALS*

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The effect produced by extirpation of the pituitary, adrenal, and pancreas on the effect of four hypoglycemic sulfonyl compounds† was studied in dogs, rats, and toads. A fall of blood sugar was observed in normal, hypophysectomized, adrenalectomized, and castrated male or female dogs. Except in a few cases, the effect could not be obtained in depancreatized dogs unless a small fragment of the organ remained.

METHODS

The substances employed were:

(1) *p*-aminobenzenesulfonamidoisopropylthiodiazole (Compound 2254 RP; IPTD; PASIT);

(2) 1-butyl-3-sulfonylurea (BZ-55, Boehringer; carbutamide, Lilly);

(3) 1-butyl-3-*p*-tolylsulfonylurea (D 860, Hoechst; Orinase, Upjohn); and

(4) *p*-aminobenzenesulfonamido-2-*tert*-butyl-5-thiodiazole (2259 RP).

The animals used were: hypophysectomized rats, dogs, and toads; adrenalectomized rats, dogs, and toads; depancreatized dogs and toads; hypophysectomized-depancreatized dogs; adrenalectomized-depancreatized and hypophysectomized-adrenalectomized dogs with partial pancreatectomy. The controls were intact animals of all three species.

The drugs were administered intravenously to dogs and rats in a dose of 0.2 gm./kg. and by the oral route to dogs (0.5 gm./kg.) and rats (0.2 gm./kg.). The toads were given the drugs (200 mg./kg.) in a volume of 2.0 ml. by dorsal subcutaneous injection. The substances were dissolved in 0.1 *N* NaOH; final concentration was 100 mg./5 ml.

The toads weighed between 150 and 210 gm., the dogs between 6 and 8 kg., and the rats between 120 and 160 gm. The rats were fasted for 5 hr. and the dogs for 18 hr. prior to the experiments. The dogs were kept on a meat diet and those that were adrenalectomized, or hypophysectomized and adrenalectomized, received 5 to 10 gm. NaCl per day and one injection of 5 mg. of cortisone acetate every 15 days when an experiment was not in progress.

The hypophysectomies were done by the parapharyngeal route in the rats, by a craniolateral approach in the dog, and by the buccal route in the toad.

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The hypophysectomized dogs were used 15 days to 3 months after the operation; the adrenalectomized dogs 4 to 15 days after the operation; and the pancreatectomized dogs 8 to 90 days after removal of the pancreas. In the triply operated dogs, 82 to 85 per cent of the pancreas was removed at the first operation. Two dogs (Nos. 77 and 78) underwent hypophysectomy 1 month later and one dog (No. 66), 7 months later. Three months after the second operation, the second adrenal was removed. The animals were maintained in perfect health using cortisone for the first week and only NaCl thereafter. These dogs suffered from weakness of the posterior extremities that could be ameliorated in a few hours by the injection of 5 mg. of cortisone. Hyperphagia and obesity developed in these animals; their weight increased by 58, 60, and 38 per cent, respectively. The completeness of the operations was verified at autopsy. The hypophysectomized dogs received an insulin sensitivity test, using 0.1 unit/kg. body weight. This produced rapid hypoglycemia and convulsions in 1 to 1½ hr., from which they could be restored by I.V. injections of glucose and food by mouth.

The toads were used 24 hr. after extirpation of the pituitary or cauterization of the adrenals. The hypophysectomized rats were used 8, 15, or 20 days after operation and the adrenalectomized animals, 5 to 15 days postoperatively. They were maintained in good condition without mortality by using a 1 per cent NaCl solution *per os* ad libitum.

Blood was obtained directly from the heart in the toads, from the tail vessels in the rats, and from the ear vessels in the dogs. The blood sugar was estimated by the Somogyi-Nelson technique.

RESULTS

Acute Intravenous Effect in Dogs

The substances were dissolved in 0.1 *N* NaOH; final concentration was 100 mg./5 ml. (2 per cent). They were injected intravenously (using the saphenous or the jugular veins) at doses of 200 mg./kg. of body weight. A similar amount of NaOH solution given alone had no significant effect in dogs and rats used as controls.

Normal dogs. At doses of 200 mg./kg. of body weight, the 4 sulfonyl compounds produced similar falls in the blood sugar level, on the average to −42 to −46 per cent of the initial level (TABLE 1, FIGURE 1). The lowest level of blood sugar was observed between 2 and 4 hr. after the injection, rising again after 6 or more hr., and returning to normal after 8 hr. or more. Most of the animals had nausea and vomiting and, between 2 and 6 hr., weakness and drowsiness; some of them lay down, and others showed tremor. Neither these symptoms nor hypoglycemia were observed after administration of NaOH (FIGURE 1).

Hypophysectomized dogs. In 14 hypophysectomized dogs, doses of 200 mg./kg. of body weight provoked in the blood sugar a slightly more accentuated descent than in the controls: an average of −50 per cent with carbutamide, −46 per cent with IPTD, and −57 per cent with 2259 RP (TABLE 1, FIGURE 1). Only one of these dogs died in hypoglycemia 24 hr. after the

TABLE 1

ACTION OF SULFONYL COMPOUNDS GIVEN INTRAVENOUSLY (200 MG./KG.) ON THE BLOOD SUGAR (MG./100 ML.) OF DOGS

Drug	Hours								Died in 24 hours
	0	0.5	1	1.5	2	4	6	8	
Normal									
Carbutamide.	99 ± 7	88 ± 10	65 ± 9	73 ± 8	57 ± 4	54 ± 6	71 ± 6	75 ± 10	0/8
Tolbutamide..	119 ± 2	96 ± 9	85 ± 8	69 ± 13	67 ± 8	78 ± 7	92 ± 4	111 ± 3	0/2
IPTD. ..	93 ± 7	76 ± 7	65 ± 4	54 ± 3	56 ± 3	52 ± 3	83 ± 1	102 ± 3	0/6
2259 RP.. . .	100 ± 7	68 ± 8	59 ± 6	—	58 ± 10	62 ± 5	68 ± 6	—	0/6
NaOH 0.1 N...	85 ± 7	90 ± 5	94 ± 5	92 ± 4	90 ± 4	81 ± 7	86 ± 7	—	0/4
Hypophysectomized									
Carbutamide. .	79 ± 4	51 ± 8	43 ± 6	40 ± 5	49 ± 4	59 ± 3	69 ± 3	—	0/5
IPTD.........	81 ± 4	59 ± 1	48 ± 2	43 ± 3	51 ± 4	61 ± 7	70 ± 4	—	0/6
2259 RP......	70 ± 2	47 ± 2	50 ± 2	41	30 ± 5	30 ± 6	42 ± 14	—	1/3
Adrenalectomized									
Carbutamide.	77 ± 9	49 ± 4	22 ± 3	16 ± 2	17	12	—	—	4/4
Tolbutamide...	91	64	58	41	37	47	—	—	1/1
IPTD.........	86 ± 6	44 ± 20	22 ± 9	13 ± 2	—	—	—	—	1/2
2259 RP	101 ± 3	68 ± 4	42 ± 7	—	24 ± 5	46 ± 4	30 ± 6	—	2/2
Adrenalectomized, Glucose									
Carbutamide...	80	200	80	175	95	80	100	—	2/4
2259 RP.......	79 ± 7	301 ± 13	184 ± 12	—	245 ± 14	212 ± 3	255 ± 10	—	2/2
Adrenalectomized, Hydrocortisone									
Carbutamide.	103 ± 11	67 ± 7	53 ± 4	46 ± 3	57 ± 9	65 ± 6	74 ± 4	84	0/3
2259 RP....	89 ± 9	47 ± 7	31 ± 10	—	49 ± 20	—	—	—	2/2
Adrenalectomized, Cortisone									
2259 RP.. . .	102 ± 15	73 ± 26	51 ± 7	—	69 ± 15	48 ± 6	70 ± 9	75 ± 15	2/2

injection of 2259 RP. In all cases, the initial blood sugar level was lower than in the normals; the maximum effect was observed 1½ to 2 hr. after the administration of the drugs; that is, more rapidly than in normals.

The usual symptoms were observed, but weakness was more intense than in normal animals. Drowsiness or even a semicomatose condition was seen in some of the hypophysectomized dogs; others showed abnormal gait or incoordination of movements, and it was difficult for them to stand. Two of these dogs had transient convulsions, and one died after a prolonged coma of 24 hr.

There is a remarkable contrast between the moderate hypersensitivity of hypophysectomized animals to sulfonyl compounds and the exaggerated hypersensitivity they show to insulin (0.1 units/kg.), which provokes severe convulsions in 1 to 1½ hr. that disappear after glucose administration.

Adrenalectomized dogs. Adrenalectomized dogs showed an extraordinary sensitivity to the hypoglycemic and toxic action of the sulfonyl compounds. The blood sugar fell rapidly, reaching very low levels after 1 to 2 hr. (TABLE 1, FIGURE 1). The animals presented tremor, prostration, intense convulsions with opisthotonos, and coma. Almost all of them died (8/9 dogs) in 2 to 4 hr. Only one dog, injected with IPTD, recovered spontaneously in 7 hr. but it died the next day. Two animals injected with 2259 RP had a transient improvement (between 2 and 4 hr.) but died 18 and 22 hr. later, respectively.

Two dogs in which the operation was not complete (a quarter of the adrenal had been left) presented intense transient symptoms after being given 2259 RP, but they recovered and survived.

Six adrenalectomized dogs injected intravenously with 200 mg./kg. of the sulfonyl compounds (4 with carbutamide and 2 with 2259 RP) received glucose treatment. The animals were given 3 gm./kg. of glucose I.V. 15 min. prior to administration of the hypoglycemic compounds and then 1.5 gm./kg. every hour thereafter. Two adrenalectomized dogs given twice-hourly glucose injections after carbutamide remained symptomless during 3 hr. Later, hypoglycemia appeared: there was an average blood sugar level of 37 mg./100 ml. in the fourth hour and 15 mg./100 ml. in the fifth. The animals died in convulsions and coma 5½ hr. after the initiation of glucose treatment.

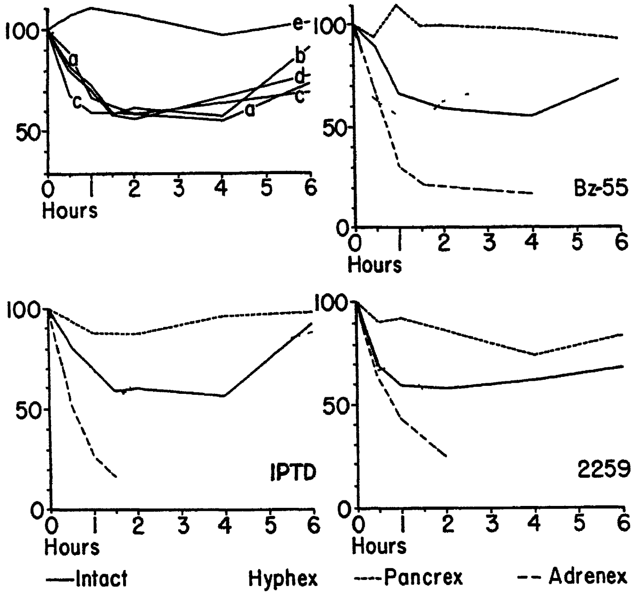

FIGURE 1. Action of sulfonyl compounds on the blood sugar level of dogs (*a*, carbutamide(BZ-55); *b*, IPTD; *c*, 2259 RP; *d*, tolbutamide; *e*, 0.1 *N* NaOH).

TABLE 2
ACTION OF SULFONYL COMPOUNDS GIVEN INTRAVENOUSLY (200 MG./KG.) ON THE BLOOD SUGAR (MG./100 ML.) OF DOGS

Drug	Hours							Died in 24 hours
	0	0.5	1	1.5	2	4	6	
				Depancreatized				
Carbutamide.	234 ± 12	217	255 ± 15	233	231 ± 3	228 ± 12	213 ± 10	0/4
IPTD .	275 ± 40	—	243 ± 31	240 ± 40	240 ± 35	258 ± 37	260 ± 39	0/2
2259 RP ...	314 ± 46	285 ± 25	289 ± 33	–	269 ± 28	234 ± 8	260 ± 35	0/3
				Depancreatized, Hypophysectomized				
Carbutamide	273 ± 24	270 ± 20	263 ± 26	267 ± 19	262 ± 25	261 ± 25	257 ± 35	0/3
Tolbutamide.	239 ± 15	229 ± 12	234 ± 11	249 ± 9	229 ± 5	238 ± 16	240 ± 10	0/3
IPTD. . .	287 ± 43	287 ± 45	286 ± 53	273 ± 38	328 ± 34	262 ± 38	254 ± 40	0/3
IPTD . .	84	—	71	62	57	49	65	0/1
2259 RP. ..	75	71	56	—	49	42	—	1/1
				Depancreatized, Adrenalectomized				
Carbutamide.	[illegible]26 ± 23	275 ± 3	247 ± 7	270 ± 4	263 ± 3	231 ± 2	223 ± 4	0/3
Tolbutamide..	293 ± 30	298 ± 32	311 ± 30	323 ± 27	343 ± 20	392 ± 7	412	3/3
2259 RP....	238 ± 70	214 ± 67	197 ± 60	—	181 ± 65	166 ± 58	161 ± 64	2/3

Two adrenalectomized dogs injected with glucose every hour for 5 hr. after carbutamide administration were symptomless, and survived. The other two injected the same way after receiving 2259 RP were also symptomless for 6 hr., but some hours later symptoms appeared, and they died between 15 and 18 hr.

Glucose tolerance curves were performed by M. Gordon in 10 adrenalectomized dogs anesthetized with chloralose (120 mg./kg.). These dogs received simultaneously glucose (3 gm./kg.) and carbutamide (100 to 200 mg./kg.). Five adrenalectomized dogs, used as controls, were given glucose alone. Ninety minutes later the blood sugar fell much more in the dogs treated with carbutamide (from 684 to 154 mg./100 ml.) than in the controls (from 880 to 335 mg./100 ml.). No significant increase in liver and muscle glycogen was observed in either group.

Corticoids had some protective action against the hypoglycemic and toxic effects of sulfonyl compounds in adrenalectomized dogs. Three dogs injected subcutaneously with hydrocortisone (5 mg./kg.) 15 hr., and ½ hr. before and 2 hr. after carbutamide administration, survived, and only one of them showed toxic symptoms (TABLE 1). Two other dogs injected with 2259 RP did not survive, in spite of receiving the same hydrocortisone treatment (TABLE 1).

Cortisone was also ineffective in preventing the death of two other adrenalectomized dogs injected with 2259 RP.

Pancreatectomized dogs. No significant fall in the blood sugar level was observed in 6 pancreatectomized dogs after injections of carbutamide and IPTD (200 mg./kg.) (TABLE 2, FIGURE 2). Insulin treatment was discontinued 24 hr. before the injections. However, a blood sugar fall (16 and 40 per cent) was observed in 2 of 3 dogs injected with 2259 RP at the same dose (TABLE 2). It is worthy of note that, in spite of the absence of hypoglycemia, pancreatectomized animals presented a toxic picture, with vomiting, abnormal gait, tremor, and difficulty in standing. Six hours later all these symptoms had disappeared.

A significant decrease in the blood sugar level was not observed in 8 hypophysectomized-pancreatectomized dogs with high initial levels injected with carbutamide, tolbutamide, or IPTD (TABLE 2, FIGURE 2).

In 2 hypophysectomized-pancreatectomized dogs, after 48 hr. fasting and with normal initial blood glucose levels, a fall in blood sugar similar to that found in normals was obtained 4 hr. after injection of 2259 RP (—44 per cent) or IPTD (—42 per cent), as shown in TABLE 2 and FIGURE 2. One of these dogs was given IPTD *per os*, 200 mg./kg./day, for 10 days before the experiment without receiving insulin; it developed anorexia and fasted spontaneously for 48 hr. No hypoglycemia was observed after a similar I.V. injection of IPTD (200 mg./kg.) in 2 pancreatectomized dogs that had not been hypophysectomized, but had received the same previous oral treatment and had a high initial blood sugar level.

The hypophysectomized-pancreatectomized dogs that had been deprived

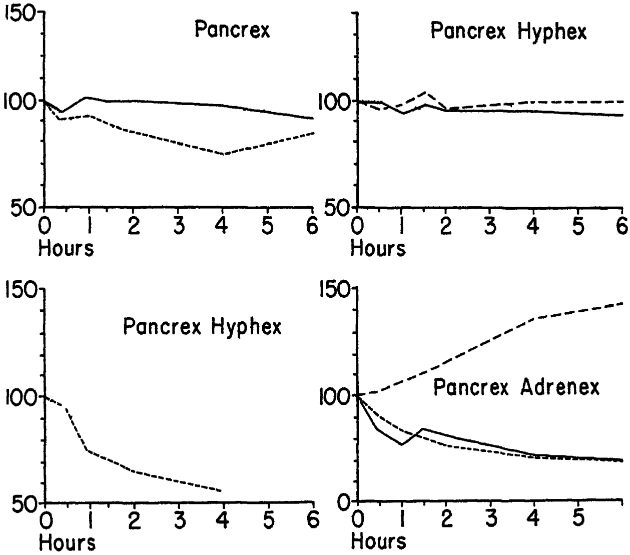

FIGURE 2. Action of sulfonyl compounds on the blood sugar of depancreatized dogs (——— carbutamide; IPTD· -- --2259 RP;—·—tolbutamide).

of food and had not received insulin showed intense nervous symptoms lasting 4 days, at the end of which they died in coma and with a blood sugar of 116 mg./100 ml. The sensitivity of these dogs to sulfonyl compounds must be attributed to the fact that they had been hypophysectomized and therefore, due to the lack of pituitary hormones and to fasting, they had normal initial blood glucose levels.

Nine pancreatectomized-adrenalectomized dogs were studied (TABLE 2, FIGURE 2). In 3 animals injected with carbutamide, a slow fall in the blood sugar, reaching a minimum (−40 per cent) 8 hr. after administration of the substance, was observed. The animals survived. A similar slow descent (−33 per cent) was observed in the 3 animals injected with 2259 RP. These 6 depancreatized-adrenalectomized dogs presented symptoms of depression and tremor, but did not have hypoglycemia and convulsions during the first hours as did the animals that were adrenalectomized only. Two of the 3 dogs injected with 2259 RP died: 1 of them 22 hr. later, after the animal had apparently recovered at 6 hr., and the other 36 hr. later, after anorexia, depression, and coma. No fall in blood sugar was found in the 3 depancreatized-adrenalectomized dogs injected with tolbutamide. One animal died 2 hr., the other two 6 hr. after injection, with the usual symptoms, but since they had received similar doses of carbutamide during the preceding days, a cumulative effect may have occurred. The blood sugar rose progressively and, when they died, marked hyperglycemia was found (412, 304, and 362 mg. per cent).

Oral Administration in the Dog

Acute experiment. Oral administration (500 mg./kg.) of any of the 4 sulfonyl compounds caused a fall in the blood sugar after 1 hr., reaching a minimum between 1 and 4 hr., and then starting to rise. Absorption was therefore rapid.

In adrenalectomized animals given carbutamide in doses of 200 and 500 mg./kg., a marked drop was observed in 1 hr. (50 mg./100 ml.); this was more marked after 1½ to 2 hr. (13 to 17 mg./kg.), and the animals died between 2½ and 3 hr.

Two pancreatectomized animals that had not received protamine insulin for 20 hr. were given 2259 RP, 500 mg./kg. Initial blood sugar levels were 409 and 400 mg./100 ml. A small drop in the blood sugar, 22 and 33 per cent respectively, was observed after 2 hr., followed by progressive recovery.

Repeated administration. Carbutamide, 500 mg./kg., was given daily to 3 dogs for 2 days. Blood glucose fell to −54 per cent on the fourth to sixth days, and then rose slowly, reaching a normal level on the eighth day.

Four hypophysectomized dogs were treated for 2 days with carbutamide (500 mg./kg./day) and for 4 days with IPTD. The decrease was similar to that of the normal animals (45 per cent), but recovery was slower—blood sugar reached a normal level only on the tenth day. The effect was, therefore, more prolonged than in the normals.

In a previous paper[1] a detailed description of four experiments in dogs was published. The animals were hypophysectomized, adrenalectomized, and partially pancreatectomized, and they developed diabetes. Carbutamide was given to 3 of them, and 1 received a single dose of IPTD, 500 mg./kg. They showed intense toxic symptoms after 4 to 5 hr., and one of them died; symptoms lasted 1 to 2 days in the other 2 dogs, in spite of high blood sugars (528 mg./100 ml. in 1 animal). In these dogs, deprived of the hypophysis and adrenals, a partial amelioration of the diabetic hyperglycemia due to the action of sulfonamides was observed. In addition, although they were not hypoglycemic, they presented nervous symptoms that were not relieved by the administration of glucose.

Prolonged administration. Four pancreatectomized dogs received IPTD at daily doses of 200 mg./kg. for 5 days. The blood sugar level of 1 animal showed no change; it rose in 2 and transiently descended in 1. In summary, there was no fall in the blood sugar level.

IPTD was administered to 2 hypophysectomized-pancreatectomized dogs at a dose of 200 mg./kg./day for 6 days. Blood sugar levels remained unchanged.

Four days later, the animals in both groups showed the following symptoms: although they were totally anorexic, they drank water, but sometimes vomited it; they had tremor, were depressed and, due to uncoordinated gait, preferred to lie down; they did not respond when called. A bloody or mucous diarrhea ensued, and soon thereafter the animals became comatose.

Autopsy was performed in 3 pancreatectomized dogs, 2 hypophysectomized-pancreatectomized, and 1 hypophysectomized-adrenalectomized-pancreatectomized dog, killed *in extremis.* Abundant mucus, spotted with blood, was found in the stomach. There were petechiae, edema of the mucosa, small superficial ulcerations in the prepyloric area and, in one of the animals, a superficial ulcer of 3 cm. diameter.

The duodenum was found to be congested, with small superficial ulcerations; the rest of the small intestine showed some congested areas and small intramucosal or submucosal hemorrhages. The large intestine showed a few punctate hemorrhages.

The thyroids of 3 pancreatectomized and 2 hypophysectomized-pancreatectomized dogs, treated with IPTD during 15, 27, 34, 40, and 55 days, weighed approximately two and a half times as much as those of controls of similar body weight.

Oral or Intravenous Administration in the Rat

In the normal albino rat, oral or I.V. administration of carbutamide, IPTD, tolbutamide, or 2259 RP, at a dose of 200 mg./kg., produced a hypoglycemic effect, with no mortality (TABLES 3 and 4). The hypophysectomized rats, injected I.V. with 200 mg./kg., showed lower blood sugar levels than the normals, with a mortality of 3/5 for each one of the two substances tested (TABLE 3). Three of the rats died between 3 and 5 hr; 3 during the night.

The adrenalectomized rats showed a marked sensitivity to the hypoglycemic and toxic effects of the sulfonyl compounds tested (TABLES 3 and 4). The lethal dose of the 4 sulfonyl compounds given per mouth was 200 mg./kg. for the adrenalectomized and 4 to 5 gm./kg. for the normals (20 to 25 times more resistant) and was found to be intermediate in adrenal-demedullated rats. When these compounds were given intravenously, the blood sugar fell to 19 to 20 mg./100 ml. in 30 min., and all the animals died with convulsions and coma, 30 min. to 1 hr. after injection (TABLE 3). Oral administration of the substances to adrenalectomized rats produced a marked drop in the blood sugar level 1 hr. later, and the animals died between 1 and 3 hr. after ingestion (TABLE 4). Before dying, they presented tremor, convulsions, opisthotonos, and coma. No changes were observed in the blood sugar levels of non-injected adrenalectomized animals after 5 hr. of observation.

Groups of rats were treated with corticoids, given subcutaneously 24 and 2 hr. before, and 5 hr. after, the oral administration of the sulfonyl compounds. Each rat received a dose of 3 mg. of cortisone acetate or 3 mg. of hydrocortisone (cortisol) in 0.5 ml. saline suspension, or 3 mg. of deoxycorticosterone (DOCA) in 0.25 ml. sesame oil. The three corticoids diminished the fall in

TABLE 3
ACTION OF SULFONYL COMPOUNDS GIVEN INTRAVENOUSLY (200 MG./KG.) ON THE BLOOD SUGAR (MG./100 ML.) OF WHITE RATS

Drug	Hours						Died in 24 hours
	0	0.5	1	2	3	5	
			Normal				
Carbutamide	103 ± 4	—	62 ± 6	—	66 ± 13	67 ± 1	0/5
IPTD	132 ± 13	—	86 ± 11	—	35 ± 6	52 ± 19	0/5
2259 RP	101 ± 6	—	72 ± 6	—	46 ± 6	60 ± 9	0/5
			Hypophysectomized 8 days				
Carbutamide	77 ± 5	—	57 ± 4	—	48 ± 6	43 ± 6	3/5
IPTD	72 ± 7	—	40 ± 6		32 ± 7	26 ± 1	3/5
			Adrenalectomized 8 days				
Carbutamide	54 ± 5	19 ± 4	—	—	—	—	4/4
IPTD	62 ± 5	20 ± 7	—	—	—	—	4/4
Tolbutamide	55 ± 3	—	—	—	—		4/4
2259 RP	64 ± 4	20 ± 6	—	—	—	—	4/4
			Adrenalectomized, Hydrocortisone				
Controls	123 ± 5	—	109 ± 4	107 ± 5	112 ± 7	113 ± 4	0/4
Carbutamide	127 ± 7	—	23 ± 2	12 ± 3	21 ± 3	48 ± 6	0/4
IPTD	119 ± 4	—	34 ± 17	20 ± 5	42 ± 14	99 ± 8	0/4
Tolbutamide	108 ± 4	—	30 ± 6	20 ± 7	30 ± 4	43 ± 7	0/4
2259 RP	106 ± 4	—	31 ± 5	20 ± 6	36 ± 8	50 ± 7	0/4

TABLE 4
ACTION OF SULFONYL COMPOUNDS GIVEN BY MOUTH (200 MG./KG.) ON THE BLOOD SUGAR (MG./100 ML.) OF WHITE RATS

Drug	Hours					Died in 24 hours
	0	1	2	3	5	
Normal						
—	90 ± 3	101 ± 3	95 ± 7	118 ± 3	86 ± 3	0/6
Carbutamide	86 ± 4	42 ± 6	25 ± 1	20 ± 2	36 ± 1	0/4
IPTD	90 ± 6	48 ± 12	23 ± 5	20 ± 4	38 ± 1	0/4
Tolbutamide	80 ± 10	23 ± 1	19 ± 1	24 ± 2	40 ± 4	0/4
Adrenalectomized						
Carbutamide	82 ± 7	31 ± 2	11	—	—	6/6
IPTD	74 ± 2	14 ± 3	—	—	—	4/4
Tolbutamide	67 ± 8	23 ± 4	6 ± 3	—	—	4/4
Adrenalectomized, Glucose						
Carbutamide	53 ± 3	33 ± 1	21 ± 1	13 ± 3	17	0/6
Adrenalectomized, Corticoids						
Carb. + hydrocortisone	127 ± 7	23 ± 2	12 ± 3	21 ± 3	48 ± 6	0/4
Carb. + cortisone	84 ± 4	44 ± 2	29 ± 2	33 ± 2	44 ± 5	3/6
Carb. + DOCA	72 ± 3	43 ± 4	31 ± 1	47 ± 6	52 ± 6	3/6
2259 + hydrocortisone	78 ± 3	43 ± 5	30 ± 2	41 ± 5	50 ± 6	0/4

blood sugar level and prevented death (TABLES 3 and 4). Cortisone and deoxycorticosterone prolonged the life of 50 per cent of the animals and saved the rest. Hydrocortisone saved all the adrenalectomized animals given any of the 4 sulfonyl compounds orally or by injection at a dose of 200 mg./kg.; in spite of having marked decreases in blood sugar, these animals recovered and survived.

Intravenous administration of glucose into the jugular veins of the adrenalectomized rats had a protective effect. These animals had an initial blood sugar level of 53 mg./100 ml. After carbutamide administration, they had intense hypoglycemia (18 mg./100 ml.) with convulsions, and died. Those given glucose (3 gm./kg.) intravenously 15 min. before administration of the drug had a blood sugar level of 33 mg./100 ml. 1 hr. later; they were then given glucose (1.5 gm./kg.) intravenously at hourly intervals for 6 hr. Two hours after carbutamide the mean blood sugar level was 21 mg./100 ml., and the rats had convulsions that disappeared after administration of glu-

TABLE 5
ACTION OF THE SULFONYL COMPOUNDS (200 MG./KG. IN 2 ML. 0.1 *N* NAOH S.C.) ON THE BLOOD SUGAR LEVEL (MG./100 ML.) IN MALE TOADS.
AVERAGES OF 6 TOADS

Drug	Hours				
	0	1	3	5	7
Normal					
—	37 ± 8	35 ± 4	41 ± 5	34 ± 2	37 ± 3
Carbutamide	35 ± 2	23 ± 2	15 ± 2	22 ± 3	39 ± 8
IPTD	45 ± 3	29 ± 3	21 ± 2	19 ± 1	39 ± 2
Tolbutamide	41 ± 2	29 ± 1	16 ± 1	11 ± 1	26 ± 1
2259 RP	39 ± 2	28 ± 3	16 ± 2	21 ± 2	41 ± 2
Adrenalectomized 24 hours					
—	29 ± 5	36 ± 3	32 ± 4	29 ± 3	34 ± 4
Carbutamide	29 ± 5	15 ± 2	1 ± 0.5	20 ± 7	18 ± 4
IPTD	29 ± 5	28 ± 6	22 ± 5	3 ± 2	13 ± 1
Tolbutamide	29 ± 5	31 ± 5	10 ± 2	3 ± 1	10 ± 1
2259 RP	34 ± 5	22 ± 7	11 ± 5	6 ± 6	25 ± 4
Depancreatized 24 hours					
—	121 ± 14	136 ± 7	128 ± 9	120 ± 11	132 ± 8
Carbutamide	135 ± 13	138 ± 22	130 ± 3	130 ± 3	136 ± 9
Adrenalectomized-depancreatized 24 hours					
—	94 ± 8	86 ± 6	90 ± 7	108 ± 3	92 ± 7
Carbutamide	94 ± 8	90 ± 9	88 ± 18	104 ± 25	113 ± 17
Adrenalectomized, hydrocortisone					
Carb. + hydrocortisone	51 ± 4	52 ± 5	50 ± 4	52 ± 5	49 ± 7
Hypophysectomized, 24 hours					
Carbutamide	40 ± 3	32 ± 2	12 ± 2	26 ± 5	24 ± 4
IPTD	40 ± 5	32 ± 5	14 ± 1	27 ± 2	35 ± 3

cose. The estimations of blood glucose were repeated after 3 hr. (13 mg./100 ml.), 4 hr. (17 mg./100 ml.), 5 hr. (17 mg./100 ml.), and 6 hr. (19 mg./100 ml.). An injection of 3 gm. of glucose in 10 ml. was made into the stomach, and all the animals survived the subsequent days.

In rats treated daily for 5 months with sulfonyl compounds, 200 mg./kg. orally, the weight of the thyroid was found to be increased by carbutamide (average 60 mg.) and not by tolbutamide (average 34 mg.); the average weight in controls was 30 mg.

Toads. The 4 drugs produced a marked hypoglycemia after injection, the lowest values being reached in 3 hr. In the hypophysectomized group the curves were similar to those found in the normal animals (TABLE 5).

As in other series, 6 to 7 hr. after the injection of carbutamide the blood sugar level in the normal group was back to the control value and in the hypophysectomized group it continued to be low.

In the adrenalectomized toads, carbutamide produced a profound and prolonged fall of the blood sugar (TABLE 5). Injection of hydrocortisone (1 mg. 15 hr. and 0.5 hr. before, and 2 hr. after, carbutamide) prevented this hypoglycemic action.

The sulfonyl compounds had no hypoglycemic action in toads depancreatized the day before (TABLE 5); the blood sugar level 6 hr. after the drug had been injected was higher than in the group not given the drug.

Summary and Conclusion

The 4 sulfonyl compounds studied (carbutamide, tolbutamide, IPTD, and 2259 RP) produced hypoglycemia in dogs, rats, and toads.

At a dose level of 200 mg./kg., the hypoglycemic effect was slightly more marked in hypophysectomized than in normal dogs, but the toxic symptoms were more intense in the hypophysectomized dogs, and 1 of 14 animals died. In the hypophysectomized rats the hypoglycemic effect was more prolonged and there was greater toxicity (6/10 deaths) than in the normal (no death in 28).

Adrenalectomized animals of all three species exhibited marked sensitivity to the hypoglycemic and toxic action of the drugs, whether injected or ingested.

The action of these substances is contrasted with that of insulin, which generally produces more intense hypoglycemia and more toxic effects in hypophysectomized than in adrenalectomized animals.

The toxic symptoms (convulsions, coma) in the adrenalectomized animals usually occurred during hypoglycemia; hourly administration of glucose improved their condition and, when repeated sufficiently, prevented death. Hydrocortisone diminished hypoglycemia in rats and toads, and prevented death of all the rats and some of the dogs. Cortisone and deoxycorticosterone saved only 50 per cent of the adrenalectomized rats.

Toxic symptoms (tremor, depression, coma, and death) were observed in adrenalectomized-pancreatectomized dogs. They were seen in animals with blood sugar levels above the normal, and were not ameliorated by glucose injections.

In depancreatized dogs and toads and in 8 hypophysectomized-depancreatized dogs, that is, in animals deprived of all pancreatic tissue, carbutamide and IPTD did not decrease the blood sugar level. Compound 2259 RP produced some decrease of blood sugar level (16 and 40 per cent) in 2 of 3 depancreatized dogs. In 2 fasted depancreatized-hypophysectomized dogs with normal initial blood sugar levels, IPTD and 2259 RP produced a decrease (−41 and −43 per cent).

In 6 of 9 depancreatized-adrenalectomized dogs rapid and intense blood sugar fall, convulsions, and death did not occur; but the animals had a slow and moderate decrease of the blood sugar level in 6 to 8 hours and, in spite of the fact that the blood sugar remained above the normal value, 5 animals died, presenting toxic symptoms.

Reference

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THE ROLE OF INSULIN IN THE ACTION OF THE HYPOGLYCEMIC SULFONYL COMPOUNDS*

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Effect of Sulfonyl Compounds on Pancreatectomized Animals

In the preceding paper it was reported that hypoglycemic sulfonyl compounds did not produce a significant decrease in the blood sugar level in the absence of the pancreas (16 depancreatized dogs and 9 hypophysectomized-depancreatized dogs). This fact was demonstrated by the intravenous injection of carbutamide (1-butyl-3-sulfonylurea, BZ-55), IPTD (isopropylsulfanylilthiodiazole, 2254 RP), and tolbutamide (1-butyl-3-tolylsulfonylurea, D 860, Orinase) in dogs totally deprived of the pancreas and in pancreatectomized-hypophysectomized animals. These sulfonyl compounds had no hypoglycemic effect on pancreatectomized toads. However, there was a fall in blood sugar (−16 and −40 per cent) in 2 of 3 pancreatectomized dogs injected with 2259 RP (*tert.*-butylsulfanylilthiodiazole); this fall can be attributed to a small amount of insulin remaining from that injected the day before or to the substance used.

A clear hypoglycemic effect was observed in 2 pancreatectomized-hypophysectomized dogs, fasting and with normal initial blood sugar levels.

In dogs deprived of the pancreas and adrenals no significant decrease was observed during the first hours; however, after 6 to 8 hours there was a decrease (average −32 per cent). All these animals presented toxic symptoms, and 5 of 9 died 22 to 36 hr. after injection of the drug. The absence of initial hypoglycemia observed is characteristic of animals deprived of the pancreas, whereas the sensitivity to the toxic effect is due to adrenalectomy.

Reinforcement of Insulin Action in Prolonged Treatment

Four pancreatectomized and 2 hypophysectomized-pancreatectomized dogs were used in these experiments. Pancreatectomized dogs were maintained after the operation with subcutaneous injections of protamine zinc insulin for 20, 34, and 37 days, respectively, until the wounds were completely healed; the animals had good appetites and were in satisfactory general condition. A uniform daily dose of protamine zinc insulin was calculated for each dog in order to maintain the blood sugar level between 200 and 300 mg./100 ml. The animals were fed boiled salted beef, 35 gm. mixed with 10 gm. of bread per kg. of body weight. Their appetite was voracious; sometimes it decreased when IPTD was given by mouth, and it always

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decreased when this substance was given without injecting insulin for 2 or 3 days.

Each of the pancreatectomized dogs received the following treatment: first, each was given a daily dose of protamine zinc insulin for at least 10 to 12 days (3 dogs received 1 unit per day, and 1 dog received 2 units per day). Then each received, associated with insulin, a daily dose of IPTD (200 mg./kg.) *per os* for 7 to 11 days. Later, the dose of IPTD was maintained, but the protamine zinc insulin was reduced to half the dose for 5 to 9 days. This treatment was repeated and, finally, IPTD was given alone for 6 to 7 days. Insulin plus IPTD was given twice to all the animals except to Dog No. 147, to which it was given 3 times (FIGURE 1).

While insulin alone maintained the blood sugar level between 200 to 300 mg./100 ml., the addition of IPTD provoked a fall to normal or hypoglycemic values (57 to 68 mg./100 ml.). This result was constant and characteristic (FIGURE 1).

When insulin was reduced to half-dose and IPTD maintained at the same dose, the blood glucose rose again, but reached a lower level than that observed with the administration of insulin alone (FIGURE 1). When insulin was discontinued and IPTD was given alone, the blood sugar level rose or remained high (FIGURE 1). The general condition of the animals became worse; one of them died in coma and the others presented anorexia and toxic symptoms. An intravenous injection of IPTD (200 mg./kg.) was given to the 3 remaining dogs before sacrifice, but no decrease in the blood sugar level was observed during 8 hours.

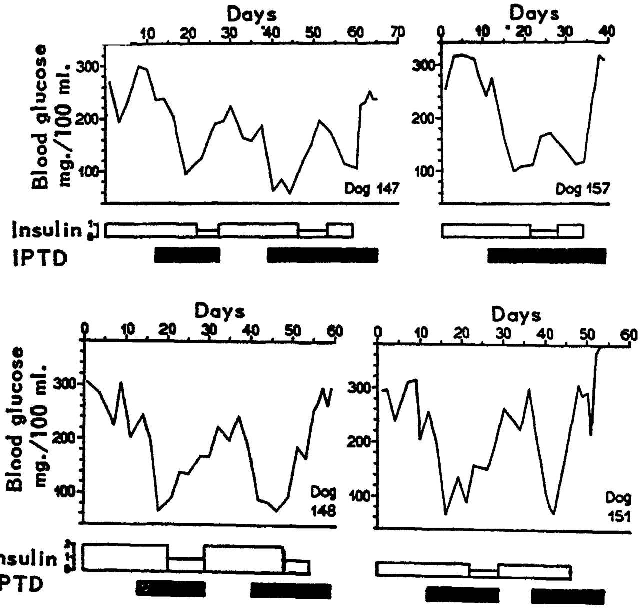

FIGURE 1. Depancreatized dogs treated with daily injections of protamine zinc insulin and with IPTD by mouth (200 mg./kg./day).

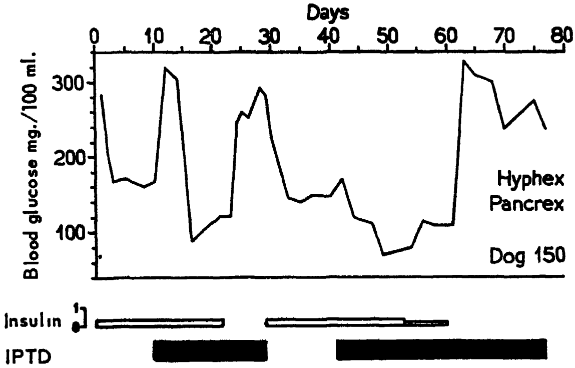

FIGURE 2. Depancreatized-hypophysectomized dog treated with daily injections of protamine zinc insulin and with IPTD by mouth (200 mg./kg./day).

The hypophysectomized-pancreatectomized dogs were given daily injections of 0.25 units and 0.30 units of protamine zinc insulin per kg. of body weight, which were sufficient to maintain their blood glucose level between 200 and 300 mg./100 ml. The addition of IPTD (200 mg./kg. by mouth) to the insulin injections produced a fall of the blood glucose to a normal level (FIGURE 2). When the dosage of insulin was reduced to a half, the blood sugar of Dog No. 150 was maintained at a normal level. When both dogs were given IPTD alone, an increase in the blood sugar was observed, and one of the animals died. In the other dog, after a 48-hr. fast, a blood sugar curve was taken: the initial blood sugar level was normal (84 mg./100 ml.); following the intravenous injection of IPTD (200 mg./kg.), a decrease of 42 per cent, similar to that in normal dogs, was observed.

These experiments were carried out by J. C. Penhos, Naide Teodosio, and J. Bowkett.

Simultaneous Injection of Insulin and Hypoglycemic Sulfonyl Compounds

Two pancreatectomized dogs received rapid injections (into different veins) of insulin (0.25 units/kg.) and carbutamide (200 mg./kg.). With insulin alone, a maximal fall of 57 and 69 per cent was observed 2 hr. later. With insulin and carbutamide there was a maximum fall of 70 and 74 per cent 2 hr. later. The blood sugar level after 6 hr. was below that seen in dogs injected with insulin alone. This experiment confirms similar results obtained by Loubatières.

Experiments with Insulin Infusion

Constant insulin infusion and a single injection of hypoglycemic sulfonyl compounds. Experiments were carried out in dogs that received a constant infusion of insulin over a 3-hr. period. Five of them were given 0.04 units/kg./hr.; 8 received 0.03 units/kg./hr. The animals were previously anesthetized with chloralose (100 mg./kg., intravenously). The experiments with 0.03 units/kg./hr. were carried out by J. C. Penhos and J. Bowkett, and those with 0.04 units/kg./hr. by R. H. Migliorini.

Three experiments were made on 3 different days on each animal: (1) rapid I.V. injection of the hypoglycemic compound, at a dose of 200 mg./kg., dissolved in 10 ml. of 0.1 *N* NaOH; (2) I.V. injection of insulin during 3 hours; (3) I.V. injection of insulin during 3 hours but, after 30 min. (Migliorini) or 50 min. (Penhos) from the beginning, the hypoglycemic substance was rapidly injected at the same dose as in the first experiment. Blood samples were collected from the ear, and blood glucose was determined by the Somogyi-Nelson method.

In the experiment in which insulin (0.04 units) and carbutamide (200 mg./kg.) were given, the results were very clear. Carbutamide alone did not cause a fall of the blood sugar level in the 5 depancreatized dogs (curve *a*, FIGURE 3). With insulin alone (0.04 units/kg./hr.) there was a moderate fall, 22 to 39 per cent (average 30 per cent) at the end of the 3-hr. infusion (curve *b*, FIGURE 3). Then the blood sugar level began to rise, reaching its initial level in about 6 hr. In the experiment in which carbutamide was injected half an hour after insulin infusion was started (0.04 units/kg./hr. during 3 hr.), the fall in the blood sugar was more marked and sustained, 46 to 68 per cent (average 59 per cent) at the end of the infusion (curve *c*, FIGURE 3). The blood sugar level began to rise, but this rise was slower than in the previous case; at the sixth hour it had not yet reached its initial level, being 13 to 39 per cent (average 36 per cent) below it.

Eight dogs received 0.03 units/kg./hr. of insulin during 3 hr. The hypoglycemic substances (200 mg./kg., I.V.) were injected 15 min. after starting the insulin infusion. The 2 dogs that received 2259 RP and 1 of the 2 dogs that received tolbutamide showed a potentiation of the hypoglycemic action of insulin. On the other hand, in 2 dogs injected with carbutamide and in 2

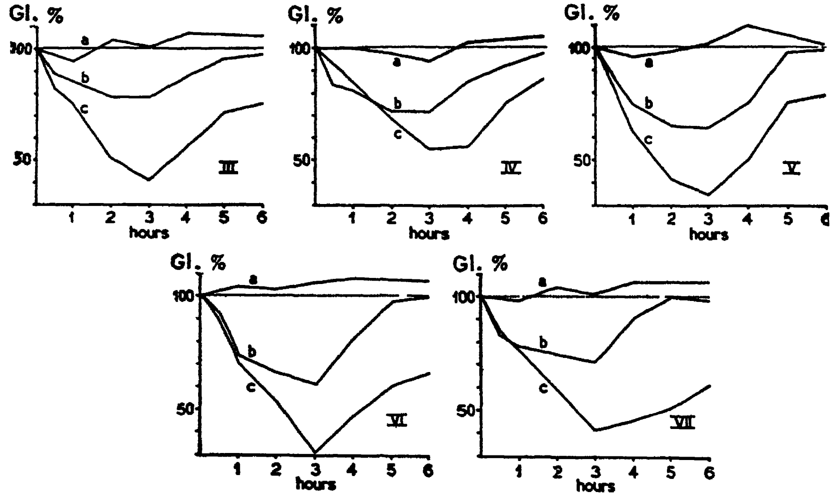

FIGURE 3. Depancreatized dogs (a) injected with carbutamide (200 mg./kg.); (b) perfusion of insulin (0.04 unit/kg./hr.) for 3 hr.; (c) same perfusion of insulin for 3 hr., with injection of carbutamide (200 mg./kg.) 30 min. after starting the perfusion.

with IPTD, no potentiation of the hypoglycemic action was observed. In summary, in only 3 of the 8 dogs did the hypoglycemic action of insulin show an increase.

Three pancreatectomized dogs received an insulin infusion of 0.05 units/kg./hr., during 3 hr. Half an hour after starting the insulin infusion, carbutamide was injected I.V. (200 mg./kg.) without observing any reinforcement of the hypoglycemic actions of insulin.

Graduated perfusion of insulin and sulfonamides. Many experiments made since 1928 have shown that the blood sugar level of the depancreatized dog may be rapidly lowered and then stabilized at a determined level when insulin is perfused first at a larger and then at a smaller dose. The initial dose may be: 0.08, 0.05, or 0.03 units/kg./hr. during 2½ hr.; then during 1½ hr., an infusion of insulin with half this dose (0.04, 0.025, or 0.015 units/kg./hr.) followed by a perfusion for a further 2 hr. with half this last dose (0.02, 0.0125, or 0.0075 units/kg./hr.). With the first dose, there is a fall in blood sugar; with the second the blood sugar level is maintained; and with the third there is a slow increase.

The rate of insulin infusion most suitable for lowering the blood sugar of each of the pancreatectomized-chloralosed dogs to approximately the normal level value was selected. The object was to obtain and maintain a level of blood sugar in which the additional effects of carbutamide were observable. This effect would be absent in cases in which the insulin infusion was insufficient or excessive. Forty-eight hours after the initial perfusion with insulin

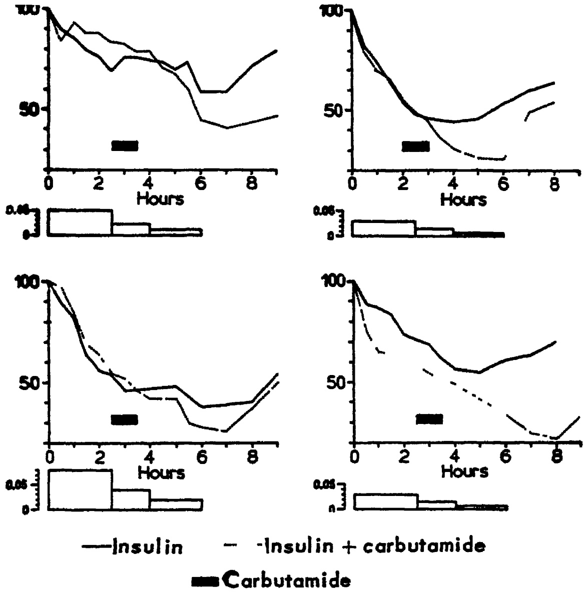

FIGURE 4 Blood sugar level of dogs under chloralose anesthesia (120 mg./kg.) receiving a constant infusion of insulin at 3 rates. The black mark indicates injection of carbutamide (200 mg./kg. in 1 hr.).

TABLE 1

Dog No	Perfusion of insulin Unit/kg./hr.			Carbutamide Mg./kg	Blood sugar		
	0 to 2½ hours	2½ to 3½ hours	3½ to 6 hours	Between 2½ and 3½ hours	Initial level Mg./100 ml	Fall %	Fall Mg
148	0.08	0.04	0.02	—	309	62	191
	0.08	0.04	0.02	200	354	74	260
155	0.05	0.025	0.0125	—	194	48	92
	0.05	0.025	0.0125	200	296	61	181
156	0.05	0.025	0.0125	—	227	58	131
	0.05	0.025	0.0125	200	286	38	108
151	0.05	0.025	0.0125	—	324	58	200
	0.05	0.025	0.0125	200	310	69	213
147	0.05	0.025	0.0125	—	224	41	92
	0.05	0.025	0.0125	200	316	59	185
156	0.03	0.015	0.0075	—	344	45	155
	0.03	0.015	0.0075	200	263	78	205
157	0.03	0.015	0.0075	—	317	56	177
	0.03	0.015	0.0075	200	335	74	246

alone, a similar one was performed, but carbutamide was injected between 2½ and 3½ hr. after starting the experiment.

In 6 of the 7 dogs, the addition of carbutamide increased the fall of blood sugar; there was only one negative result (Dog No 156). A more rapid fall in the blood sugar level was observed after injecting carbutamide (FIGURE 4 and TABLE 1). Furthermore, after 4 hr., when the animal received the third and lowest dose of insulin, the rise was slower than in the animals injected with carbutamide.

These experiments were carried out by J. C. Penhos, Naide Teodosio, and J. Bowkett.

Insulin and Hypoglycemic Sulfonamides in Eviscerated Dogs

With E. Urgoiti we carried out experiments in dogs deprived of all the abdominal viscera: digestive tract (from the anus to the cardia), urinary tract, liver, spleen, pancreas, and adrenals. The animals were under chloralose anesthesia (120 mg./kg., I.V.) and presented a good general condition until they were sacrificed 6 hr. later.

The first group of 3 controls received an infusion of insulin (0.015 units/kg./hr.) and glucose (150 mg./kg./hr.) during 6 hr. In 2 dogs, the blood

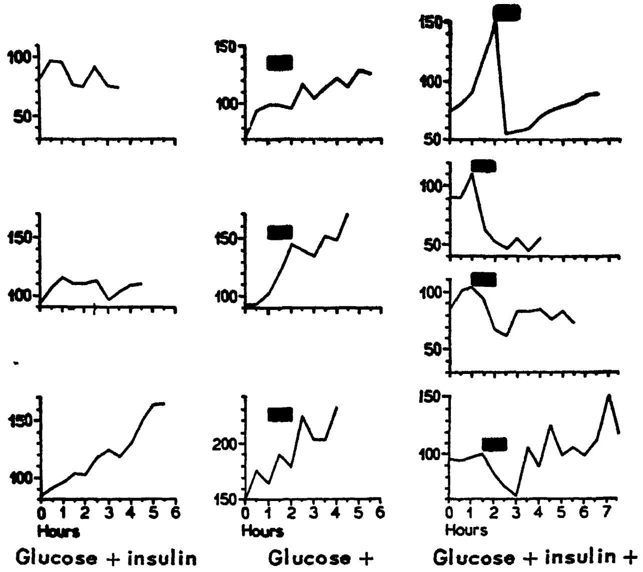

FIGURE 5. Eviscerated dogs under chloralose anesthesia (120 mg./kg.). *Left:* constant infusion of insulin (0.015 unit kg/hr.) with glucose (150 mg./kg./hr.) for 6 hr. *Middle:* Glucose (150 mg./kg./hr.) for 6 hr. and tolbutamide (100 mg./kg in 1 hr) *Right:* Insulin (0.15 unit/kg./hr.) and glucose (150 mg./kg./hr.) for 6 hr.; the mark indicates when tolbutamide was injected (100 mg./kg. in 1 hr.).

sugar level was maintained and, in the third, it increased to double the initial level (FIGURE 5).

Four dogs received the same treatment, with the addition of a perfusion of sodium tolbutamide (100 mg./kg.) from the end of the first hour to the end of the second. In all the dogs administration of tolbutamide produced a fall in the blood glucose level (36, 37, 56, and 57 per cent). This lowered level was maintained during 1, 1½, and 3 hours (FIGURE 5). The fall in the blood sugar may be attributed to the simultaneous effect of insulin and tolbutamide. The latter, by itself, did not produce a fall; on the contrary, it produced a progressive increase throughout the experiment (FIGURE 5).

In summary, the association of insulin with glucose and the association of tolbutamide with glucose did not produce a decrease in the blood sugar level at the doses employed. Tolbutamide had a hypoglycemic effect when administered with insulin and glucose. This effect was obtained in the absence of all the abdominal viscera.

Injection into the Pancreatic Artery

These experiments were carried out by E. J. Urgoiti in 65 dogs. The animals were under chloralose anesthesia (120 mg./kg.). The duodenum was ligated where it separates from the caudal end of the head of the pancreas; then the inferior pancreatic duodenal artery, a branch of the superior mesenteric artery, was ligated. The choledochus was cut; a strong ligature was placed on the hepatic pedicle, leaving out the portal vein. The splenic artery was tied near the pancreas; all the vascular branches going to the stomach and the left coronary gastric artery were also tied.

An extremely thin polyethylene catheter was inserted into the splenic artery up to the origin of the hepatic artery. The solution was infused through this catheter at a rate of 5 ml./kg., in 10 to 20 min. (average 15 min.) and mixed with the blood circulating through the hepatic and splenic arteries, going only to the pancreas, pylorus, and duodenum, and returning to the portal vein.

Glucose levels were determined in the carotid blood prior to laparotomy, at the beginning and at the end of the infusion, and after ½, 1, 1½, 2, 3, 4, 5, and 6 hr.

Many experiments were done using sodium tolbutamide dissolved in distilled water (*p*H 7.3). Other series were done with IPTD, carbutamide, and tolbutamide dissolved in 0.1 *N* NaOH.

Insertion of the catheter alone produced no significant variations in the blood glucose level; the drops seen were never greater than 10 to 16 per cent (FIGURE 7).

Sodium tolbutamide solutions were neutral (*p*H 7.3). The doses injected varied between 2.5 and 100 mg./kg. of body weight. The largest number of experiments was done with 12 mg./kg. (FIGURE 6). In addition to the dogs in which the substances were injected into the pancreatic artery, others received the substances into the saphenous and/or the portal vein.

As can be seen in FIGURES 6 and 7, no significant differences were observed between the fall of blood sugar in dogs injected into the pancreatic artery or the saphena; these experiments did not demonstrate a stronger effect in those animals injected into the pancreatic artery.

Experiments carried out with substances dissolved in NaOH (0.1 *N*) were not conclusive because the NaOH alone produces a marked fall in blood sugar, although this is slightly less than that produced by sulfonyl compounds (FIGURE 7). Although alkaline sulfonyl solutions produced greater descents

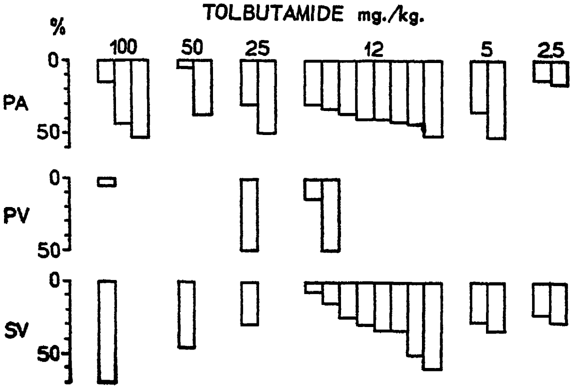

FIGURE 6. Fall of blood sugar level (mg./100 ml.) in dogs under chloralose anesthesia (120 mg./kg.). PA, arterial intrapancreatic injection of tolbutamide; PV, portal vein injection; SV, saphenous vein injection.

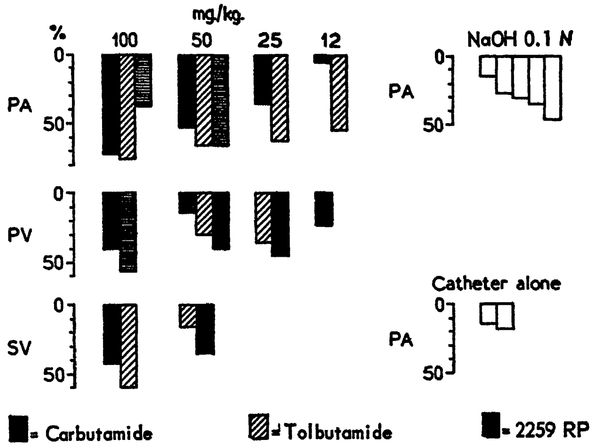

FIGURE 7. Fall of blood sugar level (mg./100 ml.). PA, arterial intrapancreatic injection of sulfonyl compounds dissolved in 0.1 *N* NaOH; PV, portal vein injection; SV, saphenous vein injection.

when injected into the pancreatic arterial blood than when they were injected into the saphena or the porta, it was not possible to assert statistically that these substances stimulate the pancreas specifically.

Summary and Conclusions

(1) It was reported in a previous paper that in 16 depancreatized dogs and toads and in 9 hypophysectomized-depancreatized dogs—that is, in animals deprived of all pancreatic tissue—the injection of hypoglycemic sulfonyl compounds did not decrease the blood sugar level, but some decrease (16 and 40 mg. per cent) was observed in 2 of 3 totally depancreatized dogs injected with 2259 RP. In 2 fasting depancreatized-hypophysectomized dogs with normal initial blood sugar levels, the injection of IPTD and 2259 RP produced a typical hypoglycemia.

(2) In depancreatized-adrenalectomized dogs the compounds did not decrease the blood sugar in the first hours as in the normal dogs, but there was a decrease (average −32 per cent) after 4 to 6 hr. The toxic symptoms were marked, and 5/9 dogs died before 24 hr.

(3) A series of experiments was carried out in which carbutamide was administered orally for many days to 4 depancreatized and 2 hypophysectomized-depancreatized dogs. The blood sugar level maintained by insulin (200 to 300 mg./100 ml.) was decreased to normal or subnormal levels by the oral administration of IPTD. With smaller doses of insulin, the effect was incomplete and, without insulin, the IPTD alone was unable to control the rise of blood sugar. The reinforcement of the action of insulin by IPTD was constantly obtained 9 times in the 6 dogs.

(4) In 2 depancreatized dogs the fall obtained by injection of insulin and carbutamide was greater than that obtained with insulin alone.

(5) Intravenous infusion of insulin was performed in 14 depancreatized

dogs (under chloralose anesthesia) during 3 hr. The same experiment was repeated and injections of carbutamide (200 mg./kg.) were made 30 min. after the beginning. In 5 dogs perfused with 0.04 units/kg./hr. of insulin, carbutamide reinforced the hypoglycemic action of insulin given alone, but in animals perfused with 0.03 units/kg./hr. of insulin, it increased the insulin hypoglycemia in only 3 of 8 animals.

(6) Perfusions of insulin were done in depancreatized dogs (chloralose anesthesia). The rate of perfusion was established so as to obtain an initial rapid decrease of blood sugar to the normal level and to maintain it at this level. The initial dose was 0.08, 0.05, or 0.03 units/kg./hr. during 2½ hr.; then, for the next 1½ hr., these doses were reduced to one half and the perfusion maintained; later, these doses were again reduced to one half, and the blood sugar began to rise slowly.

The experiment was repeated 48 hr. later, but from 2½ to 3½ hr. after the beginning of the experiment carbutamide (200 mg./kg.) was perfused by another vein. In 6 of 7 cases, the fall in blood sugar was more intense when carbutamide was perfused during the insulin action.

(7) When eviscerated dogs were perfused for 6 hr. with glucose (150 mg./kg./hr.) and insulin (0.015 units/kg./hr.), the blood sugar level was maintained or increased. During perfusion of glucose and injection of sodium tolbutamide (100 mg./kg. in 1 hr.) the blood sugar rose and no hypoglycemic action was observed. However, in eviscerated dogs perfused with glucose and insulin, injection of sodium tolbutamide produced a blood sugar fall.

(8) All these experiments show that the hypoglycemic sulfonyl compounds are active in the presence of insulin in depancreatized or eviscerated dogs. The action is obtained only with sufficient dosage and duration of action of insulin, and only when there is an appropriate relation between the dosages of insulin and the sulfonyl compounds.

(9) The experiments with intrapancreatic (arterial) injection of sulfonyl compounds were not conclusive.

THE EFFECT OF TOLBUTAMIDE ON INSULIN-I^{131} DEGRADATION IN THE EXTRAHEPATIC TISSUES*

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The physiological significance of the degradation of insulin-I^{131} that is observed after its injection in animals is not known. However, the high rate of destruction of insulin-I^{131} (and presumably of insulin) that has been reported strongly suggests that the rate of insulin degradation may contribute, under some conditions, to the diabetic state in man.[1] It is well established that tolbutamide and carbutamide inhibit insulin-I^{131} degradation *in vitro*;[2, 3] however, the significance of these results with respect to the intact animal has been questioned.[4] Although much information has been provided by Mirsky, Williams and their co-workers and by others regarding the degradation of insulin-I^{131}, most of the detailed studies have been either with the intact animal or with isolated kidney and liver systems. In this paper no attempt will be made to review the extensive literature pertaining to insulin-I^{131} degradation. However, it may be stated that, for all practical purposes, no data are available for the extrahepatic tissues. In a previous paper[5] we reported on the validity of insulin-I^{131} studies in the eviscerated rabbit preparation. These studies included the effect of carrier on insulin-I^{131} degradation and the relationship between circulating insulin-I^{131} and biological activity. It was concluded from these studies that the system that degrades insulin-I^{131} does not distinguish between insulin-I^{131} and natural insulin. Similar conclusions had been reached by other workers employing other test preparations. We showed also that there was a close relationship between circulating insulin-I^{131} and biological activity. Since the above results indicated that studies with insulin-I^{131} were of real value in our test preparation, we have extended these studies to include the effect of tolbutamide on insulin-I^{131} degradation.

In this study two types of rabbit preparations were employed. In some experiments the animals were eviscerated, but the kidneys were left intact. Our previous work had shown that the kidneys appeared to be the most important site of insulin-I^{131} degradation. In other experiments eviscerated-nephrectomized animals were used. These animals have a much slower rate of insulin-I^{131} degradation. The insulin-I^{131} degradation was measured from the circulating plasma by the usual 10 per cent trichloroacetic acid (TCA) precipitation procedure. In this procedure it is assumed that the insulin-I^{131} is precipitated by TCA and that the degraded products are TCA-soluble. The details of the experimental procedures have been reported elsewhere.[5]

In TABLE 1 are data showing the effect of tolbutamide on insulin-I^{131}

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The insulin used in this work was contributed by the Lilly Research Laboratories, Indianapolis, Ind.

TABLE 1
EFFECT OF TOLBUTAMIDE ON INSULIN-I^{131} DEGRADATION IN EVISCERATED-NEPHRECTOMIZED RABBITS
Results Expressed as Percentage of Total Plasma Radioactivity Not Precipitable by Trichloroacetic Acid

Hours	Insulin-I^{131} (0.2 unit/kg.)		Insulin-I^{131} + 200 units/kg. Insulin	
	Control Exp. 583	Tolbutamide* Exp. 588	Control Exp. 608	Tolbutamide* Exp. 609
0 5	16	12	5	8
1	33	30	11	14
2	58	53	26	24
3	71	75	32	35
4	75	79	44	43

* Tolbutamide (250 mg./kg.) was given intravenously as the Na salt 15 minutes before the administration of the insulin-I^{131}.

TABLE 2
EFFECT OF TOLBUTAMIDE ON DEGRADATION OF INSULIN-I^{131} WITH CARRIER INSULIN IN EVISCERATED RABBITS WITH INTACT KIDNEYS
Results Expressed as Percentage of Total Plasma Radioactivity Not Precipitable by Trichloroacetic Acid

Hours	Insulin-I^{131} + Insulin (200 units/kg.)	
	Control	Tolbutamide*
	Exp. 610	Exp. 611
0 5	15	15
1	35	44
2	65	70
3	76	79
4	78	80

* Tolbutamide (250 mg./kg.) was given intravenously as the Na salt 15 minutes before the administration of insulin-I^{131} and natural insulin.

degradation in eviscerated-nephrectomized animals. In experiment 583 and 588 physiological amounts of insulin-I^{131} (0.2 unit/kg.) were administered intravenously. The tolbutamide (exp. 588) was administered at the relatively high dosage level of 250 mg./kg. about 15 minutes before the administration of insulin-I^{131}. It is apparent from the data that tolbutamide does not alter the rate of insulin-I^{131} degradation.

Experiments 608 and 609 were carried out in a similar fashion except that natural insulin (200 units/kg.) was added as carrier to the insulin-I^{131}. Although the rate of degradation is decreased to a considerable degree by the presence of the carrier insulin, no lowering in the degradation rate of the insulin-I^{131} was observed in the presence of tolbutamide.

In TABLE 2 are data obtained with eviscerated animals with intact kidneys. Natural insulin as carrier was added to the insulin-I^{131}. Again it is apparent that the administration of tolbutamide does not influence the rate of insulin-I^{131} degradation.

Summary and Conclusions

The effect of tolbutamide on insulin-I^{131} degradation has been examined, using the eviscerated-nephrectomized rabbit and the eviscerated rabbit with intact kidneys.

The administration of tolbutamide intravenously 15 minutes before the injection of insulin-I^{131} did not influence the rate of insulin-I^{131} degradation.

From these studies we conclude that it is unlikely that the hypoglycemic action of tolbutamide can be explained by an inhibition of the mechanism that degrades insulin.

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THE RESPONSE OF KIDNEY, LIVER, AND PERIPHERAL TISSUES TO TOLBUTAMIDE AND INSULIN*

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Little or nothing is known about the physiological activities of the sulfonylurea compounds other than their hypoglycemic effect. In the course of recent investigations it was found that both tolbutamide (Orinase) and insulin produce a marked depression of phosphate excretion by the kidney. In addition, it was found that tolbutamide depresses liver function in the dog, as measured by bromsulphalein (BSP) clearance; no comparable effect was observed in the normal, diabetic, or cirrhotic patient. Finally, as has been reported previously, tolbutamide does not influence peripheral glucose utilization in normal or diabetic patients.[1] In the present paper, these findings are presented in the reverse order.

Effect of Tolbutamide on Peripheral Glucose Utilization

Insulin increases the utilization of glucose by peripheral tissues, and a technique that adequately demonstrates this action in the human has been reported from this laboratory.[2] The effect of tolbutamide in this experimental system was studied in 13 normal and in 6 diabetic patients. No increase in A-V/A† values was observed following tolbutamide,[1] whereas a significant increase in this measurement did occur after insulin.[2]

In another group of studies, 7 mild diabetics were given tolbutamide orally for 10 to 14 days following a control period of 7 to 14 days on constant diet alone.[1] All patients responded by a fall of blood sugar to or toward normal level and by a sharp reduction in glycosuria. Oral glucose tolerance tests with simultaneous arteriovenous glucose and serum (venous) phosphorus determinations were done before, during, and after tolbutamide administration. Tolbutamide did not alter the shape of the glucose tolerance or phosphorus curves; these remained diabetic in type. Nor did tolbutamide affect the arteriovenous glucose differences significantly.

The results of both the acute and prolonged experiments are considered to indicate that tolbutamide has no enhancing (insulinlike) effect on the peripheral utilization of glucose in normal or diabetic subjects. The possibility that tolbutamide causes the release of endogenous insulin is not excluded by these findings. It is conceivable that the action of exogenous insulin differs somewhat from that of the endogenous hormone by reason of its preparation and/or route of administration.

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* Supported by a grant from The Upjohn Company, Kalamazoo, Mich.

† Arteriovenous glucose difference arterial glucose concentration. It has been shown that A-V/A values are an adequate index of the rate of peripheral glucose utilization under the experimental conditions employed here.[3]

Effect of Tolbutamide on Bromsulphalein Clearance

During the course of experiments in the unanesthetized trained dog, a decreased bromsulphalein (BSP) clearance by the liver was noted following intravenous tolbutamide.[1] A summary of these data is given in TABLE 1. The decrease in BSP clearance occurred in all animals tested and was of considerable degree; the mean increase in portal-vein plasma BSP level following tolbutamide was 124 per cent, with a range of 59 to 202 per cent. Control experiments in 2 animals using sulfadiazine instead of tolbutamide revealed no significant change in portal-vein BSP levels (−2 per cent and +3 per cent respectively). These results suggested that tolbutamide causes an impairment of liver function in the dog. Accordingly, similar studies were carried out in 3 groups of patients: (1) 12 men without clinical or laboratory evidence of diabetes or liver disease; (2) 6 diabetic patients; and (3) 8 patients with clinical and laboratory evidence of cirrhosis of the liver.

All subjects were tested under standardized basal conditions in a special room after a fast of 12 to 14 hours. An intravenous loading dose (100 or 200 mg.) of BSP was administered, followed by a constant infusion (Bowman pump) of BSP in 5 or 10 per cent dextrose for a period of 70 to 130 min.

TABLE 1
THE EFFECT OF TOLBUTAMIDE ON PLASMA BROMSULPHALEIN (BSP) LEVELS IN THE DOG

Experiment No.	BSP level (mg. %)		Per cent change in BSP level
	Control	After tolbutamide	
1	2.11	3.36	+59
2	1.72	2 87	+67
3	1.17	2 63	+125
4	1.78	4.47	+151
5	1.40	4.23	+202
6	1.50	3 38	+125
7	1.25	2.79	+181
8	1.75	4.32	+147
9	0.58	0.94	+ 62
10	1.28	2.78	+117
	Control	After sulfadiazine	
11	0.94	0.92	− 2
12	1.50	1.55	+ 3

Plasma samples were obtained from the portal vein (London cannula). BSP was infused at a constant rate throughout all experiments. The values given are averages of all control and experimental levels, respectively, corrected for minor changes in BSP infusion rate.

TABLE 2

THE EFFECT OF TOLBUTAMIDE ON PLASMA BSP LEVELS IN THE HUMAN

Subject	BSP load Mg.	BSP infusion Mg./min.	Plasma BSP levels Mg. % Time (min.)												
			10	20	30	40	50	60	70	80	90	100	110	120	130
Normals															
VS......	200	4.4	4.0	2.9	2.3	2.3	2.5†	2.4	2.3	2.4	2.3	2.4	2.4	2.4	2.4
PR......	200	4.7	2.5	1.1	1.1	0.7	0.8†	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7
JA.....	200	4.7	2.0	1.0	0.8	0.8	0.8	0.8†	0.7	0.7	0.7	0.7	0.6	0.6	
RS......	200	4.9	2.1	1.2	1.0	0.8	0.9†	0.9	0.9	0.9	0.9				
WT.....	160	4.4	1.8	1.1	1.1	1.0	1.1†	1.0	1.1	1.0	1.0	1.0	1.0	1.0	
HC......	200	4.8	4.6	3.4	2.6	2.4†			1.9	1.9	2.0	2.0	2.0	2.0	2.0
LJ.....	200	3.1	0.6	0.6	0.7	0.6	0.5	0.5†		0.5	0.5	0.5	0.5	0.5	0.5
JTC.....	200	3.2	7.5	4.7	2.6	1.3†	1.0	1.0	0.8	0.7	0.3	0.0	0.0		
JTC*....	200	3.1	2.1	0.9	0.6	0.6	0.7	0.7	0.7	0.6	0.6	0.6	0.6		
JC1. ..	200	5.0	2.6	1.5	0.8	1.1	1.0	1.0†	1.0	1.0	0.8	1.0	0.9		
CT*....	200	3.5	1.5	0.5	0.5	0.4	0.4	0.4	0.3	0.3	0.3	0.2	0.1		
EB*....	200	3.8	10.7	8.9	2.9	2.0	2.0	1.6	1.6	1.5	1.4	1.3	1.0		
DB......	200	5.2	2.5	2.6	3.0	2.8	2.6†	2.6	2.6	2.5	2.5	2.4	2.4		
Diabetics															
JR......	200	3.4	5.0	3.2	1.1	1.0	0.6	0.7†	0.3	0.7	0.5	0.5	0.2	0.0	
HB......	200	3.7	12.6	4.0	1.1	0.5†	0.4	0.4	0.4	0.2	0.0	0.0	0.0	0.0	
RS....	200	5.25	4.6	3.4	1.8	2.7†	2.7	2.8	2.7	2.9	2.9	3.0	3.3		
EP.....	200	3.1	1.8	1.2	0.9	1.0	1.0†	1.0	1.1	1.1	1.1	1.2	1.3		
LM.....	200	4.5	1.9	1.1	0.9	0.8	0.9	0.9†	0.8	0.7	0.7	0.7			
EH......	200	3.1	1.2	0.9	0.8	0.8	0.7	0.7†	0.3	0.4	0.4	0.4			
Cirrhotics															
JW......	100	3.1	10.1	7.5	5.0	3.3	3.1	3.5†	3.6	3.7	3.9	4.3	4.0	4.7	
JW*.....	100	3.1	17.2	13.0	8.8	5.3	4.1	4.1	4.1	4.2	4.4	4.5	4.5	5.0	
FD......	100	5.2	4.0	4.1	4.4	4.7	5.3	6.1	6.6†	7.2	7.5	7.5	7.5	7.5	
FD*.....	100	5.0	11.7	9.0	6.3	5.2	5.5	6.3	7.0	7.1	7.1	8.3	8.9	9.9	
GK.....	200	3.4	4.2	1.0	0.5	0.7†	0.7	0.0	0.0	0.0	0.0	0.0			
SM......	145	3.2	7.0	6.9	6.7	6.2†	5.7	6.8	6.7	7.4	7.3	7.4	7.3	6.8	
FP.. . .	200	2.5	4.3	3.9	3.6†	3.5	3.4	3.5	3.5	3.7	3.8	3.9	4.0	4.1	
FP*.....	200	2.7	4.2	4.3	4.4	4.6	5.1	5.5	6.3	6.0	6.2	6.4			
V... ...	200	3.7	6.2	4.1	3.5†	3.4	2.9	3.1	3.3						
WH... .	200	3.5	4.0	2.0	1.7	2.0	2.1	2.1†	2.1	2.2	2.3	2.4	2.1	1.9	
HW. ...	200	3.2	4.0	3.6	3.7	3.8	3.9	3.9†	3.9	3.9	4.1	4.0	4.1	4.3	4.4

Note the relative constancy of venous plasma BSP levels in all patients, whether or not tolbutamide was administered, in contrast to the findings in the dog (TABLE 1). The elevated initial values (first 30 min.) are due to the loading dose of BSP.

* Control experiment: no tolbutamide administered.

† Administration of tolbutamide (Orinase Sodium) intravenously.

Blood samples for glucose and BSP analysis were drawn through an indwelling needle (antecubital vein) at 10-min. intervals, 30 to 60 min. before, and 40 to 90 min. after a single intravenous dose (1.5 to 2.5 gm.) of Orinase Sodium.* Blood sugar determinations were performed in duplicate

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* Kindly supplied by C. J. O'Donovan, The Upjohn Company, Kalamazoo, Mich.

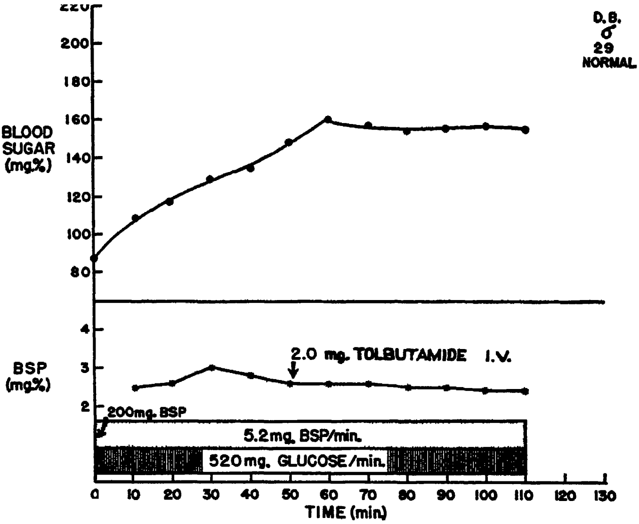

FIGURE 1. The effect of tolbutamide on plasma BSP in a normal patient. Note the constancy of the BSP level following tolbutamide. The hypoglycemic effect of the tolbutamide is partially offset by the high glucose infusion rate.

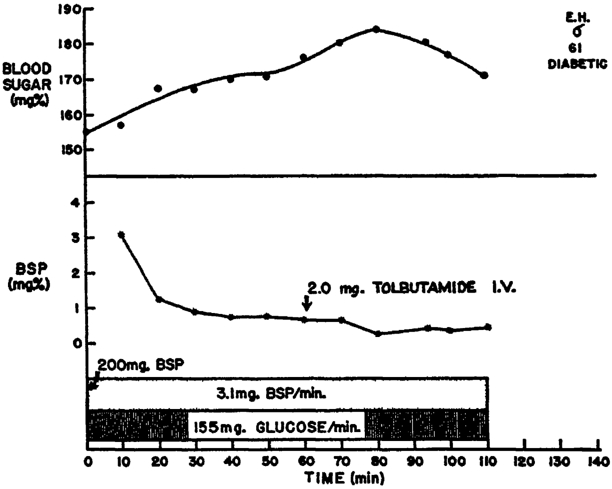

FIGURE 2. The effect of tolbutamide on plasma BSP in a patient with diabetes. Note the lack of change in BSP level following tolbutamide.

by the Nelson-Somogyi method.[4] Plasma BSP levels were determined spectrophotometrically.[5]

The data on all cases are summarized in TABLE 2. Examples from each of the three groups of patients are shown in FIGURES 1, 2, and 3. Six control experiments in which no tolbutamide was administered were done. The results in all subjects studied were similar: plasma BSP levels remained essentially constant. The data indicate that tolbutamide does not influence

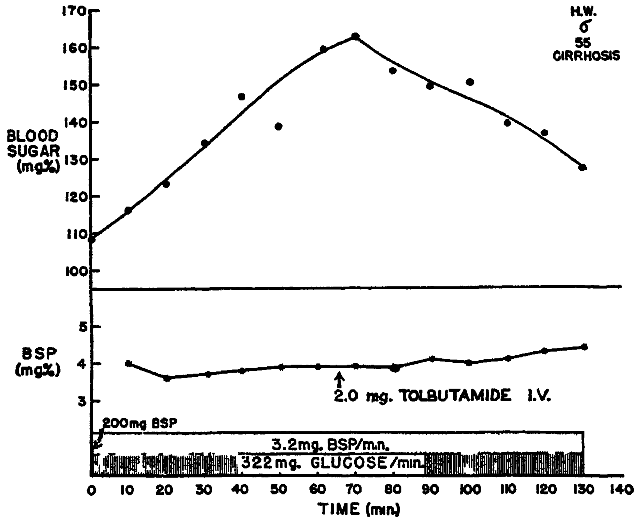

FIGURE 3. The effect of tolbutamide on plasma BSP in a patient with cirrhosis Note the absence of effect of tolbutamide on BSP clearance even in the presence of well-marked cirrhosis of the liver (evidenced by the persistently elevated BSP levels). The blood sugar response to tolbutamide is marked.

the clearance of BSP in man, even in the presence of well-marked cirrhosis of the liver. The lack of agreement between dog and human data is probably due to a species difference in response to or in metabolism of tolbutamide. It may be that in the dog tolbutamide and BSP compete with each other for biliary excretion. Such a competition has been observed between BSP and decholin.[6]

Effect of Tolbutamide on Renal Excretion of Electrolytes

The action of tolbutamide and insulin on the renal clearance of electrolytes was studied in 5 nondiabetic patients with normal renal function. Standard renal clearance techniques with 10-min. urine collection periods were employed. The subjects were given a liter of water at the onset and frequent additional water during the experiment to maintain adequate urine flows (7 to 14 ml./min.). Each experiment consisted of 3 periods before, and 4 to 7 periods following, the administration of tolbutamide or glucagon-free insulin.* In 3 subjects insulin was added to the constant infusion of normal saline in a dose of 0.1 units/min.; in 2 subjects tolbutamide (Orinase Sodium) was administered intravenously in a dose of 1.5 or 2 gm. over the course of 5 or 6 min. Sodium and potassium were analyzed with the Beckman flame photometer, chloride by the method of Van Slyke and Hiller,[7] creatinine by the Jaffe reaction,[8] and inorganic phosphate by the method of Fiske and SubbaRow.[9]

The data from these studies are given in TABLE 3, and typical experiments

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* Kindly supplied by O. K. Behrens and W. R. Kirtley of the Eli Lilly Co., Indianapolis, Ind.

TABLE 3
THE EFFECT OF TOLBUTAMIDE AND INSULIN ON RENAL CLEARANCE OF ELECTROLYTES

Subject	Insulin units/min.	Orinase gm.	Measurement	Urine						Plasma				
				Urine flow	C_{Cr} ml./min.	C_P C_{Cr} %	C_K C_{Cr} %	C_{Na} C_{Cr} %	C_{Cl} C_{Cr} %	Glucose mg. %	P‡ mg. %	K‡ mEq./l.	Na‡ mEq./l.	Cl‡ mEq./l.
RR	0	1.5	Control*	12.2	123.1	2.6	25.4	0.9	1.3	87.2	2.8	5.0	150.0	106.0
			Experimental†	13.2	131.7	0.9	21.5	0.8	1.1	42.8	1.6	3.9	152.0	108.0
			Per cent change	+8.2	+7.0	−65.4	−15.4	−11.1	−15.4	−51.0	−42.9	−22.0	−1.3	+1.9
LH	0	2.0	Control	12.9	119.8	8.4	8.2	1.0	1.2	107.5	3.2	4.8	144.0	106.0
			Experimental	13.6	117.9	1.6	9.4	1.2	1.2	65.7	2.7	4.4	144.0	111.0
			Per cent change	+5.4	−1.6	−80.9	+14.6	+20.0	0.0	−38.9	−15.6	−8.4	0.0	+4.7
WH	0.1	0	Control	9.9	88.8	10.6				87.6	3.2			
			Experimental	10.8	95.9	1.7				55.1	2.1			
			Per cent change	+9.0	+8.0	−84.0				−37.1	−34.3			
FM	0.1	0	Control	7.5	112.9	10.0				86.7	3.1			
			Experimental	13.4	122.1	3.0				55.8	2.6			
			Per cent change	+78.7	+8.2	−70.0				−35.6	−16.1			
LS	0.1	0	Control	8.6	109.2	13.1	10.8	0.5	0.6	93.6	3.3	4.5	144.0	104.0
			Experimental	13.3	116.9	5.7	13.3	0.4	0.7	52.8	2.6	4.2	138.0	102.0
			Per cent change	+54.6	+7.1	−56.5	+23.1	−20.0	+16.7	−43.6	−21.2	−6.6	−4.2	−2.0

Symbols: C_{Cr} = creatinine clearance; C_P C_{Cr} = percentage of filtered load of inorganic phosphate excreted; C_K C_{Cr} = percentage of filtered load of potassium excreted; C_{Na}/C_{Cr}. = percentage of filtered load of sodium excreted; C_{Cl} C_{Cr}. = percentage of filtered load of chloride excreted; P, K, Na, and Cl: plasma levels of inorganic phosphate, potassium, sodium, and chloride.

The increase in urine flow is an artefact due to induced water diuresis, which is part of the clearance technique; this increase occurs regularly.

* Values are average of the 3 control periods.

† Values are average of the 3 experimental periods corresponding to the periods of maximum hypoglycemia (except for plasma, P, K, Na, and Cl: see‡).

‡ The experimental value for these electrolytes is the value that shows the greatest change from control levels.

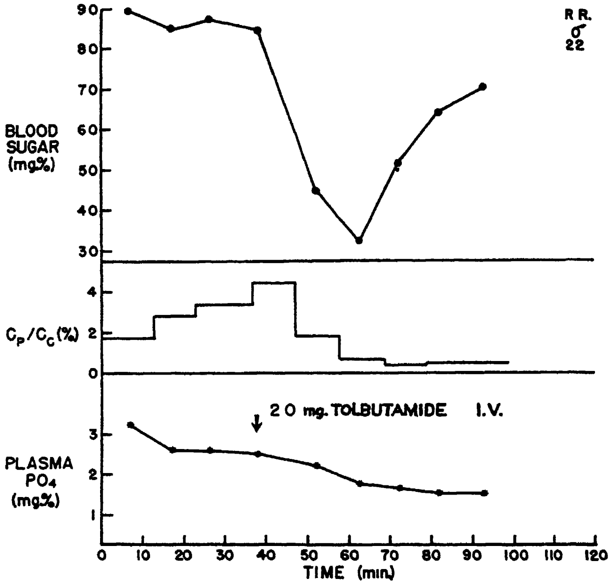

FIGURE 4. The effect of tolbutamide on phosphate clearance. Note the striking fall in phosphate clearance and the decrease in serum phosphate level.

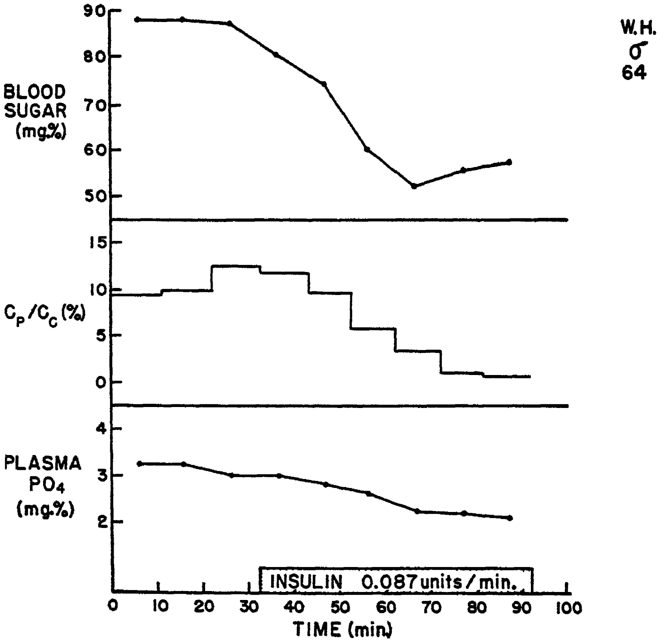

FIGURE 5. The effect of insulin on phosphate clearance. Note the parallelism between blood sugar and phosphate clearance, as well as the fall in serum phosphate level.

are shown in FIGURES 4 and 5. Both insulin and tolbutamide resulted in a striking reduction of phosphate clearance, a fall in plasma phosphate and potassium, and inconsistent or minor changes in clearances of sodium, chloride, potassium, and creatinine. None of the subjects exhibited clinical signs or symptoms of hypoglycemia, although blood sugar levels fell to a range of 32 to 59 mg. per cent.

The results show that insulin and tolbutamide have similar effects on the renal clearance of electrolytes and on plasma electrolyte levels. The fact that creatinine clearance remained constant indicates that the marked effect on phosphate excretion is not due to changes in renal hemodynamics. Studies from this laboratory have shown that renal phosphate clearance exhibits a significant positive correlation with blood glucose level.[10] In addition, recent experiments indicate that the decrease in phosphate clearance is due to the fall in blood glucose rather than to insulin *per se*, and that the serum phosphate fall is a relatively unimportant factor in the decreased phosphate clearance.[11]

Concluding Remarks

Studies on the action of tolbutamide (Orinase) on peripheral glucose utilization, on hepatic BSP clearance, and on the renal clearance of electrolytes in normal, diabetic, and cirrhotic patients have been described. The data show that tolbutamide does not resemble exogenous insulin in its effect on peripheral glucose utilization, whereas it does simulate insulin action on the kidney. The impaired BSP clearance by the liver, previously reported in the dog, was not observed to occur in man. It is concluded that the action of the hypoglycemia-producing sulfonylurea compounds is complex and that results obtained in one species cannot be assumed to be valid in another.

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RESPIRATORY QUOTIENT AND NITROGEN BALANCE DURING TOLBUTAMIDE ADMINISTRATION*

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The basic question concerning oral sulfonylureas effective in diabetes remains unanswered—do they lower blood sugar by restoring metabolism toward normal in various ways, as does insulin, or is this blood sugar effect the result of some essentially unphysiological action? Acute studies of the effects of tolbutamide (Orinase), mostly in normal subjects, have shown changes in capillary blood sugar differences and in venous potassium and inorganic phosphorus concentrations much like those following the injection of insulin.[1] The present paper describes a study of two other indices of metabolism, respiratory quotient and nitrogen balance, in two diabetic patients given tolbutamide in rather large doses for about two weeks. The patients contrasted with each other in their response to the drug; one showed a satisfactory drop in urine and blood sugar, the other did not.

Methods

Both patients, hospitalized on the metabolism ward, ate a diet of constant carbohydrate, protein, and fat composition (carbohydrate, 210 gm.; protein, 85 gm.; and fat, 70 gm.; yielding 1810 calories). Urine was collected in 24-hour samples and was analyzed for sugar by Benedict's method and for nitrogen by a micro-Kjeldahl method. Accuracy of collection was checked by creatinine measurement. Fecal nitrogen was not measured, but was assumed to represent a constant proportion (10 per cent) of dietary nitrogen. Capillary blood sugar was measured before breakfast and supper by the Somogyi-Nelson colorimetric method.

Respiratory quotients (R. Q.) were measured by the general technique described by Peters and Van Slyke.[2] The measurements were done at 8:00 A.M., after a 10-hour fast. Expired air was collected in a Tissot spirometer; carbon dioxide and oxygen were measured in duplicate with the Scholander apparatus, on duplicate or triplicate samples from the spirometer. Carbon dioxide and oxygen contents of expired air were also recorded continuously by means of the Liston-Folb infrared carbon dioxide analyzer and the Beckman paramagnetic oxygen analyzer. The results checked well with the Scholander values.

Results

Case 1. Mrs. H. P. (No. 892869) is 43 years old. Diabetes began 5 years ago, with typical symptoms of thirst, frequent urination, and weight loss, and has been controlled only moderately well by diet and daily doses of 60 units of

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NPH insulin. Her height is 65 inches and she weighs about 110 lb.; she looks thin. She has never been in frank acidosis.

Insulin was gradually reduced until urine sugar reached a value of at least 50 gm. a day; the dose was then held constant at 30 units daily for the rest of the study. Blood sugar showed wide and variable daily swings, from 10 to 150 mg. per cent in a day. After 12 days at this insulin dose, tolbutamide (Orinase; U-2043) was given by mouth in divided doses, 5 gm. a day. After 9 days there was no apparent effect on urine or blood sugar, and the dose of tolbutamide was raised to 10 gm. a day for another 4 days, still without any consistent effect on urine or blood sugar. No acetone appeared in the urine.

Nitrogen balance did not change appreciably during tolbutamide administration; if anything, it became slightly negative. However, before tolbutamide the patient was approximately in nitrogen balance—not in marked negative or positive balance.

The total respiratory quotient also did not change during tolbutamide administration; possibly there was a slight and temporary drop after the initial dose of 2 gm. Before tolbutamide the respiratory quotient was 0.80, close to a normal value.

TABLE 1

FASTING RESPIRATORY QUOTIENTS IN DIABETIC SUBJECTS IN RELATION TO TOLBUTAMIDE TREATMENT

Case 1 (Mrs. H. P.)

Day of study		O_2 consumed Liters per minute	CO_2 produced Liters per minute	Total respiratory quotient
	Before tolbutamide			
21		0.168	0.135	0.80
22		0.157	0.127	0.81
24		0.180	0.144	0.80
	On tolbutamide			
24	1 hour	0.180	0.142	0.79
28	4 days	0.165	0.134	0.81
31	7 days	0.172	0.134	0.78

Case 2. (Mrs C. S.)

	Before tolbutamide			
13		0.201	0.161	0.80
	On tolbutamide			
20	6 days	0.220	0.177	0.80
27	13 days	0.216	0.173	0.80
41	*13 days after tolbutamide*	0.215	0.168	0.78

Each value represents the mean of duplicate analyses with the Scholander apparatus of duplicate or triplicate samples from the spirometer.

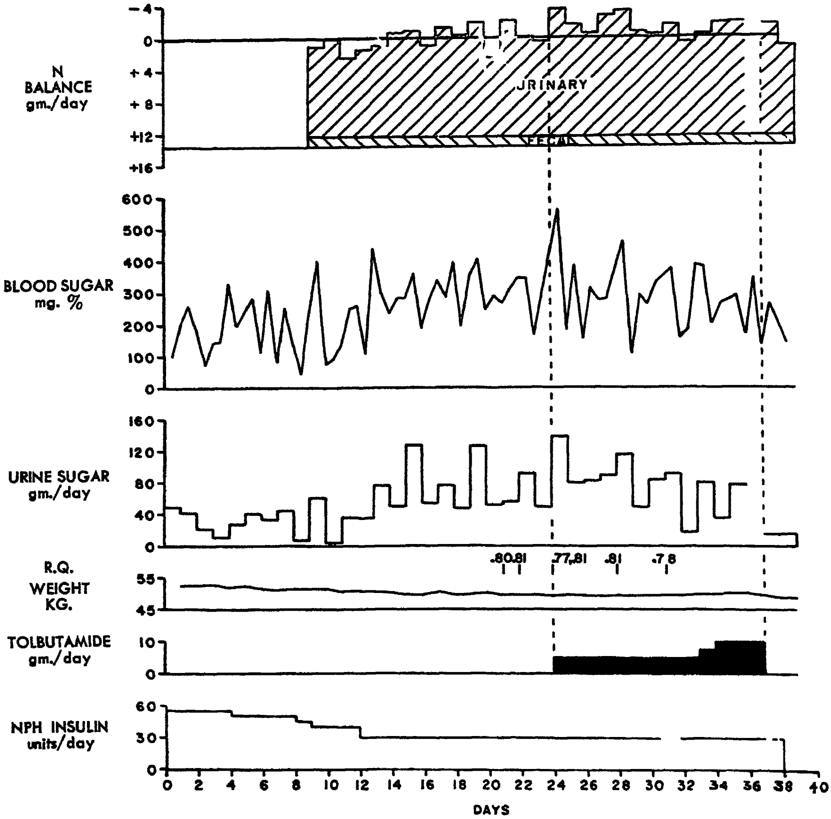

FIGURE 1. Effects of tolbutamide on nitrogen balance, blood and urine sugar, and respiratory quotient in a diabetic patient (Case 1). Nitrogen balance is plotted according to Albright's[3] method: when the hatched column extends above the zero line, balance is negative; when it is below the zero line, balance is positive.

Data for the respiratory quotients are given in the first part of TABLE 1; all the data are plotted in FIGURE 1.

Case 2. Mrs. C. S. (No. 855096) is 62 years old. Her diabetes was discovered 3 years ago by chance; she did not have typical symptoms. She had never taken insulin. Her urine has steadily shown increasing amounts of sugar. She is 62 inches tall and weighs about 160 lb.; she is moderately fat.

In the control period, urine sugar remained between 15 and 30 gm. a day and blood sugar between 245 and 300 mg. per cent. When tolbutamide administration was begun at a dose of 5 gm. a day, urine sugar promptly dropped to less than 5 gm. a day; blood sugar fell gradually, reaching normal levels of 98 to 140 mg. per cent only when the tolbutamide dosage was raised to 10 gm. a day. After 15 days tolbutamide administration was discontinued. During the following 3 weeks, blood sugar rose slowly, while urine sugar remained negligible (less than 2 gm. a day). These discrepancies suggest

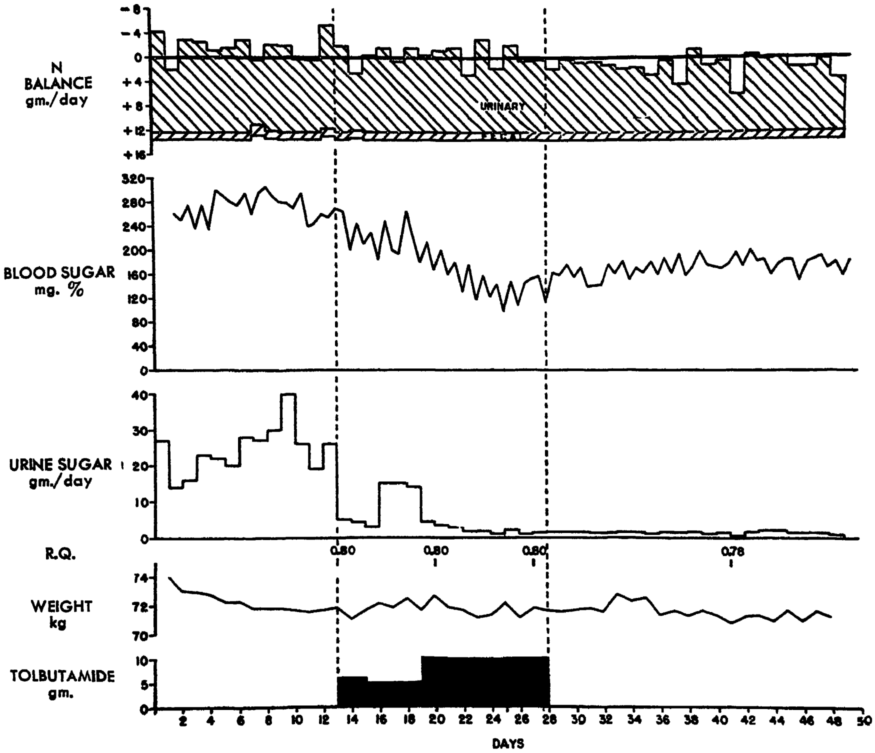

FIGURE 2. Data from Case 2, plotted as in FIGURE 1.

an effect of tolbutamide on the renal threshold for glucose. No acetone appeared in the urine at any time.

Nitrogen balance changed very little before, during, or after tolbutamide; the patient remained essentially in balance throughout.

The respiratory-quotient data are given in the second part of TABLE 1; all the data are plotted in FIGURE 2.

Comment

It is clear there was no appreciable change in nitrogen balance or in respiratory quotient when either patient was given tolbutamide. This was true whether or not there was a response of blood and urine sugar to the drug. The tentative conclusion may be drawn that, when given for several weeks, tolbutamide does not alter carbohydrate metabolism sufficiently to give an over-all increase in R. Q. or to produce a positive nitrogen balance, even when an effect on urine and blood sugar has been produced.

However, it must be noted that neither patient was in really negative nitrogen balance before tolbutamide was given, and that the R. Q. before tolbutamide was nearly normal and not the classic low R. Q. of "total diabetes." This is presumably because it was necessary to give the first patient some insulin by injection to avoid ketosis; the second by nature did not tend toward marked abnormality (some endogenous insulin available?).

Possibly a patient who is more completely "diabetic" initially would be a more favorable subject for a demonstration of the effectiveness of tolbutamide. However, present experience indicates that such a patient would be unlikely to respond to the drug even in terms of blood and urine sugar levels.

These results do not accord with the acute studies mentioned earlier, which imply an effect of tolbutamide on the peripheral utilization of sugar. It is possible that the acute and chronic effects of tolbutamide depend on different mechanisms: the acute fall in blood sugar on insulin release from the pancreas; the chronic effects on a hepatic action. Alternatively, the chronic release of insulin under the influence of tolbutamide might simply be too mild an effect to be detected by the methods used here.

Acknowledgment

My thanks for the sugar determinations are due Valerie Josephson. The respiratory quotients were measured in the Department of Physical Medicine, University Hospitals, Minneapolis (F. J. Kottke, director); I am particularly grateful to Jean Danz for her help with them.

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STUDIES OF THE EFFECT OF INTRAVENOUS TOLBUTAMIDE ON PYRUVIC AND LACTIC ACID CONCENTRATIONS IN PERIPHERAL VENOUS BLOOD IN NORMAL AND DIABETIC SUBJECTS, AND ON SPLANCHNIC METABOLISM OF FRUCTOSE AND GLUCOSE*

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Part I. Comparison of Effects of Insulin and Tolbutamide on Concentrations of Glucose, Pyruvic Acid, and Lactic Acid in Peripheral Venous Blood

Introduction

It has been known for some time that a blood sugar depression following the administration of insulin may be accompanied by concomitant elevations in blood pyruvate and blood lactate levels.[1, 2, 3] Moorhouse and Kark[4] have reported that the blood sugar depression following tolbutamide administration in a diabetic is not accompanied by these changes in pyruvate and lactate; and Hennes *et al.*[5] have reported that in normal subjects the initial pyruvate change associated with hypoglycemia is an elevation following insulin, but a depression following tolbutamide. From this disparity the latter authors concluded that the hypoglycemic actions of the two agents are distinct and that a release of endogenous insulin is not the mechanism of action of tolbutamide. It was our objective to produce with insulin and tolbutamide (Orinase§) blood sugar depressions similar in magnitude and rate of fall and, under these conditions, to compare the pyruvate and lactate changes in both normal and diabetic subjects.

Methods

The comparative insulin and tolbutamide response tests were performed in each of 4 normal and 12 diabetic subjects. All tests were done in the morning after a 12- to 14-hour fast and at the time of the test the diabetics were free of exogenous insulin effect. Two grams of Orinase Sodium diluted to 50 ml. in normal saline were administered intravenously over a 2-minute period in both groups of subjects. The normal subjects received from 3 to 5 units of regular insulin by vein. The diabetics were given their insulin subcutaneously, the dose varying from 10 to 30 units according to an estimate of their

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† Charles O. Finley Research Scholar of the American College of Surgeons.

‡ Trainee, National Institute of Arthritis and Metabolic Diseases.

§ Kindly supplied by The Upjohn Company, Kalamazoo, Mich.

insulin sensitivity based on their most recent maintenance dose This selection of the subcutaneous route for insulin administration to the diabetic subjects was in line with the objective of producing blood sugar depressions similar to those produced by tolbutamide For with tolbutamide we have not, in diabetics, produced the sharp early depression of blood sugar that may be seen after the intravenous administration of insulin. All subjects were recumbent for an hour prior to the start of the tests. The blood specimens were drawn through an indwelling Riley needle, and analyses were made according to the following methods: glucose by the method of Somogyi,[6] pyruvic acid by the method of Friedemann and Haugen;[7] and lactic acid by the method of Barker and Summerson [8]

Results

FIGURE 1 and TABLE 1 illustrate the mean blood glucose, pyruvate, and lactate changes seen in the normal subjects after the administration of tolbutamide and insulin. Comparison of the blood glucose curves reveals essentially similar curves, the minimum level following tolbutamide being at 45 min., that following insulin, at 30 min. However, a statistical comparison of the curves reveals that the apparent lag in the blood sugar depression following tolbutamide is real, within a probability of less than 0.02. Tolbutamide produced a blood sugar depression of 51 mg./100 ml./hr.; after insulin the mean rate of blood sugar depression was 70 mg./100 ml./hr.

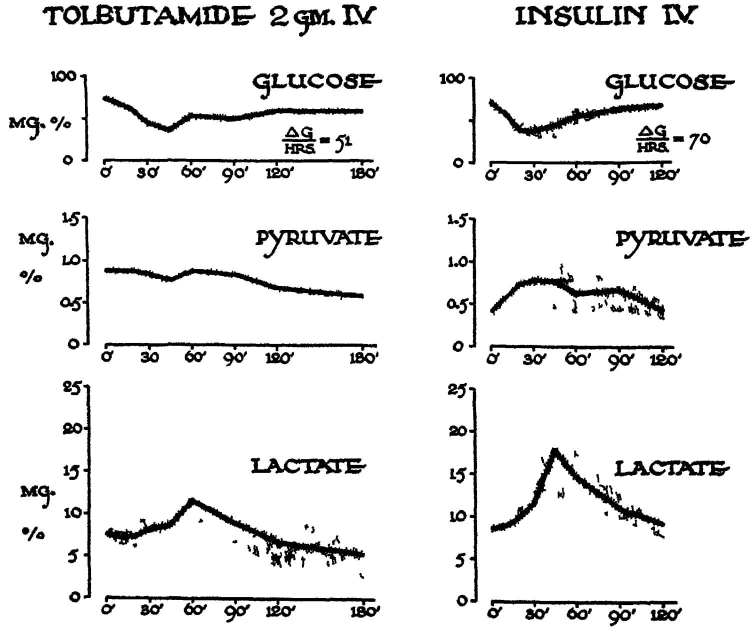

FIGURE 1 Mean changes in blood glucose, pyruvate, and lactate following tolbutamide and insulin administration to 4 normal subjects The shaded portions indicate plus or minus one Standard Deviation of the change from the fasting value in mg. per 100 ml

TABLE 1
MEAN CHANGES IN BLOOD GLUCOSE PYRUVATE AND LACTATE IN 4 NORMAL SUBJECTS FOLLOWING TOLBUTAMIDE AND INSULIN

A Tolbutamide	2 gm I V	F	10	20	30	45	60	90	120	180 min
Glucose	Mg change S D	74 +9 5	−5 +3 6	−14 +2 5	−29 +4 8	−38 +10 0	−23 ±7 6	−25 +16 5	−15 +8 2	−15 ±2 3
Pyruvate	Mg change S D	0 8) +0 31	−0 01 +0 11	−0 02 +0 21	−0 05 +0 31	−0 13 +0 32	−0 01 +0 27	−0 06 +0 30	−0 22 +0 30	−0 30 ±0 28
Lactate	Mg change S D	7 6 ±0 86	−0 5 +0 72	−0 2 +1 43	+0 6 +2 14	+1 0 +1 06	+4 0 +4 57	+1 3 ±2 18	−1 0 +2 88	−2 3 ±2 74
B Insulin	I V	F	10	20	30	45	60	90	120	min
Glucose	Mg change S D	71 +5 1	−13 +2 4	−32 +8 8	−35 +9 1	−23 +15 6	−16 ±9 3	−8 +2 4	−3 ±6 5	
Pyruvate	Mg change S D	0 41 +0 14	+0 17 +0 12	+0 33 +0 14	+0 38 ±0 28	+0 37 ±0 32	+0 21 ±0 27	+0 26 ±0 22	+0 04 +0 09	
Lactate	Mg change S D	8 5 +1 42	+0 4 +0 55	+1 4 +1 07	+3 0 +1 42	+9 4 +1 24	+6 1 ±2 81	+2 5 +1 64	+0 7 +1 57	

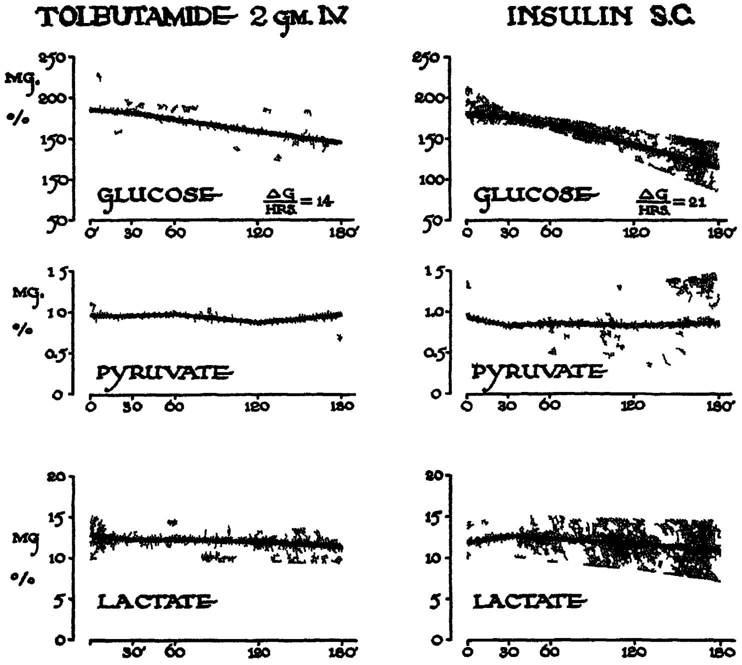

FIGURE 2 Mean changes in blood glucose, pyruvate and lactate following tolbutamide and insulin administration to 12 diabetic subjects The shaded portions indicate plus or minus one Standard Deviation of the change from the fasting value in mg per 100 ml

The mean pyruvate response after the tolbutamide tests showed no significant change from the initial level. The pyruvate curve following insulin administration was suggestive of a response, but it was only the value at 20 min. that, within the conventional confidence limits of 0.05, was significantly different from the fasting level.

However, as the mean fasting level in the insulin experiments was lower than the mean fasting value in the tolbutamide experiments, the rise observed, which brought the pyruvate levels only into the same range, may be of limited metabolic significance.

The peak values in the blood lactate levels observed following tolbutamide and insulin administration occurred in both instances 15 min. later than the maximum blood sugar depressions and probably reflect an epinephrine response to the symptomatic hypoglycemia experienced by the normal subjects in 7 of the 8 total tests.

FIGURE 2 and TABLE 2 give the mean blood glucose, pyruvate, and lactate values observed in the diabetic subjects before and after the administration of tolbutamide and insulin. The blood glucose depression following tolbutamide was 14 mg./100 ml./hr.; that following insulin was 21 mg./100 ml./hr. A comparison of the pyruvate and lactate values following the administration of these two hypoglycemic agents reveals no significant change from the initial values for either substance.

TABLE 2

MEAN CHANGES IN BLOOD GLUCOSE, PYRUVATE, AND LACTATE IN 12 DIABETIC SUBJECTS FOLLOWING TOLBUTAMIDE AND INSULIN

A. Tolbutamide	2 gm. I V	F	30	60	120	180 min
Glucose	Mg. % change S D.	186 ±54 6	−5 ±13 0	−13 ±18 4	−28 ±30 0	−41 ±38 8
Pyruvate	Mg. % change S.D.	0 96 ±0 47	−0 01 ±0 22	+0 02 ±0 26	−0 10 ±0 37	0 00 ±0 38
Lactate	Mg. % change S.D.	12 8 ±3 01	−0 6 ±1 76	−0 6 ±2 57	−0 8 ±2 40	−1 6 ±1 91
B. Insulin	S C.	F	30	60	120	180 min
Glucose	Mg. % change S.D.	180 ±35 4	−3 ±8 4	−11 ±7 6	−36 ±18 9	−64 ±30 7
Pyruvate	Mg. % change S.D.	0 94 ±0 62	−0 11 ±0 32	−0 07 ±0 38	−0 10 ±0 51	−0 08 ±0 61
Lactate	Mg. % change S.D	11 9 ±2 77	+0 6 ±2 35	+0 4 ±2 82	−0 2 ±3 08	−1.0 ±3 84

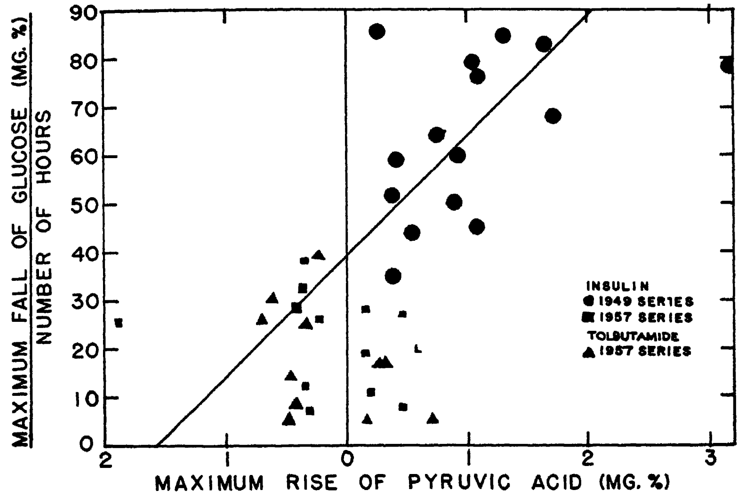

FIGURE 3. The relation between blood glucose and pyruvic acid after administration of insulin and tolbutamide to diabetic subjects. The insulin was administered intravenously in the 1949 series (circles). The line of least squares is derived from these points. In the 1957 series insulin was given subcutaneously (squares). The rate of glucose depression was slower; the pyruvate changes were small and variable in direction. Similar glucose and pyruvate responses were obtained with tolbutamide (triangles).

In FIGURE 3 the individual maximum rises in blood pyruvate observed following tolbutamide and insulin administration to the diabetic subjects are plotted against the associated rates of blood sugar depression expressed in mg./100 ml./hr. Included also are the results from a previous study of pyruvate response following *intravenous* administration of insulin to diabetic subjects.[3] The line of least squares is derived from these latter points. It intersects a zero change in pyruvate at 39 mg./100 ml./hr. blood sugar fall. It will be noted that the points representing the present experiments with both tolbutamide and insulin (subcutaneous) fall below this rate of blood sugar depression (mean values 14 and 21 mg./100 ml./hr., respectively). The individual pyruvate changes are seen to be of small magnitude and of no consistency as regards positive or negative direction of change—for either the tolbutamide or insulin experiments.

Discussion

Our results fail to establish a clear difference between the hypoglycemias following tolbutamide and insulin administration in either normal or diabetic subjects, using as indicators the two intermediates in glucose metabolism, pyruvate and lactate. Particularly in the diabetic subjects, where the rate of blood sugar depression was slow, the changes in pyruvate and lactate were small and variable in direction for both the hypoglycemic agents. The report of Hennes *et al.*[5] is concerned with a comparison of tolbutamide and insulin effects in normal subjects only. It is important to note also that their conclusion regarding different mechanisms of hypoglycemic action is based upon the initial deflections of blood pyruvate. Thus, following insulin administration, they reported an increase in pyruvate as the earliest significant change associated with hypoglycemia in 7 of the 8 experiments. Following tolbutamide, they found the earliest significant change to be a decrease in pyruvate in 5 of 7 experiments.

Analysis of our results with regard to the *initial* pyruvate change reveals that in the 4 normal subjects there was after insulin administration an increase in all the experiments; following tolbutamide, 2 subjects showed increases and 2 decreases. In the 12 comparative experiments on diabetic subjects, the earliest pyruvate change associated with hypoglycemia after insulin was an increase in 7 instances, a decrease in the remaining 5. After tolbutamide administration, the same subjects showed 5 increases as the initial pyruvate change, and 7 decreases. Our results, then, confirm the previous report with regard to the initial pyruvate change that may be expected after insulin administration to normal subjects. The initial change in pyruvate after tolbutamide administration to normal subjects, and after either agent to diabetic subjects, was variable in direction.

From our studies we find ourselves in agreement with the previous reports[4, 5] regarding the absence of increases in pyruvate and lactate incident to tolbutamide administration. We are less sure that the rises reported after insulin administration are indicative of differing mechanisms of action. It may be more likely that the differences in pyruvate response reflect differences in rate of glucose fall. Differing mechanisms of action may exist, but we are doubtful that changes in blood pyruvate and lactate are the proper indicators to establish the point.

Summary

(1) Hypoglycemic responses to both insulin and tolbutamide were produced in each of 4 normal and 12 diabetic subjects, and the accompanying blood pyruvate and lactate changes were observed.

(2) In the normal subjects a significant rise in pyruvate was associated with the hypoglycemic response to insulin administration; the pyruvate changes following tolbutamide were variable in direction, and there was no significant mean change from the initial level.

(3) In the diabetic subjects the rates of glucose depression following both insulin and tolbutamide were less than those seen in normal subjects, and there was no significant change from the fasting value in either pyruvate or lactate following either hypoglycemic agent.

(4) It is concluded that a mechanism of action for tolbutamide distinct from that of insulin cannot be inferred from differential responses of the blood pyruvate and lactate. Such a differential was not observed in diabetic subjects. It is suggested that the differences observed in normal subjects may be related to differing rates of glucose fall.

Addendum

Since preparation of this paper, the work of Hennes *et al.* has been published in more extended form.[9] In their normal subjects, despite the use of approximately one half the amount of Orinase Sodium we employed, they had a mean rate of blood sugar depression of about twice what we observed following tolbutamide administration to normal subjects. Hennes *et al.* performed their pyruvate determinations according to the chromatographic method of Seligson and Shapiro,[10] which gives generally lower values than the method of Friedemann and Haugen,[7] which we used. An inspection of Hennes' data shows that unfortunately his normal subjects, as did ours, had lower fasting pyruvate levels before the insulin tests than before the tolbutamide tests (means of 3.8 and 5.1 μM./100 ml. respectively). Hennes suggests as a possible alternative to his conclusion that tolbutamide might produce an initially increased rate of pyruvate removal simultaneous with, and independent of, stimulation of insulin secretion. The variability in the direction of the initial change in both our normal and diabetic groups makes us unable to support this hypothesis.

Other contributors to this publication have raised the possibility of differing mechanisms of action for exogenous and endogenous insulin. Perhaps herein will lie the explanation for such differences in pyruvate and lactate responses to insulin and tolbutamide as may ultimately be established.

Part II. Effect of Tolbutamide on Splanchnic Metabolism of Fructose, Glucose, Pyruvic Acid, and Lactic Acid During Fructose Administration

Introduction

It has been reported that in man the rise in the peripheral venous blood glucose concentration that accompanies the administration of fructose or galactose is diminished or abolished by the sulfonylureas. On the contrary, the changes in the blood glucose concentration that are associated with glucose administration are not influenced by these drugs.[4, 11, 12] Since all or most of the conversion of fructose to blood glucose occurs in the splanchnic system, these findings suggested that the sulfonylureas had an effect upon splanchnic carbohydrate metabolism. Hepatic vein catheterization studies have been performed in human subjects to investigate more directly this possible mechanism of action of tolbutamide.

Methods

The subjects for these studies were 5 diabetic and 2 nondiabetic patients. In all 5 cases the diabetes mellitus was of the stable type. One patient was receiving 65 units of insulin daily, 3 were receiving 15 units daily, and the fifth patient did not require insulin. On another occasion, the effect of tolbutamide on the peripheral venous blood glucose concentration of the diabetic subjects was tested by administering a single intravenous dose of 2 gm. of the sodium salt of the drug. A significant decline in the blood glucose concentration was produced in 4 cases; the patient who required 65 units of insulin daily did not respond to the test dose of sodium tolbutamide, and the data from the hepatic vein catheterization study in this patient were not included in the final compilation of results. The diabetic patients were given only regular insulin for 1 to 3 days before the hepatic vein catheterization study and none on the morning of the test, so that no exogenous insulin effect was present at the time of the procedure. The tests were performed after an overnight fast. After fasting blood samples had been obtained, an infusion of 10 per cent fructose* in water was started in a peripheral vein and continued throughout the remainder of the test at the rate of 1 gm. per kg. per hr. At an average time of 48 min., after the beginning of the infusion, 2 gm. of sodium tolbutamide was injected into a peripheral vein during a 2-min. period, and the study was continued for an average of 55 min. longer. At 9- to 12-min. intervals throughout the test, blood samples were obtained simultaneously through a radiopaque catheter with its tip located in a right hepatic vein and through an indwelling needle in a radial artery. These blood samples were analyzed for fructose, glucose, and pyruvic and lactic acids by the methods described previously.[13] Splanchnic blood flow was estimated by the bromsulphalein technique.[14] The estimated net splanchnic assimilation of a substance was calculated by multiplying its arterial-hepatic venous blood concentration difference by the estimated splanchnic blood flow. Three additional subjects—2 diabetic and 1 nondiabetic—served as controls. Control tests were performed in an identical manner except that an injection of saline was substituted for the sodium tolbutamide.

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* Fructose solutions were supplied by Mead Johnson & Company, Evansville, Ind.

Results

As seen in FIGURE 4 and TABLE 3, when fructose was administered there was a prompt uptake of this hexose by the splanchnic system. The rate of uptake was similar in diabetic and nondiabetic patients, in accord with our previous observations.[13] The magnitude of the uptake increased during the early part of the experiment and was then maintained during the remainder of the test. Tolbutamide did not appear to alter the rate of the splanchnic uptake

TABLE 3
EFFECT OF TOLBUTAMIDE ON MEAN NET SPLANCHNIC ASSIMILATION OF FRUCTOSE, GLUCOSE, AND PYRUVIC AND LACTIC ACIDS DURING FRUCTOSE ADMINISTRATION
(Mg./min./m.^{2})

Tolbutamide Series (6 cases)

Time (min.)	0	0 to 48	49 to 103
Substance administered	—	Fructose	Fructose + Tolbutamide
Fructose	5	370	439
Glucose	−28	−104	−15
Pyruvic acid	0.4	−9.8	−15
Lactic acid	13	−20	−61

Control Series (3 cases)

Time (min.)	0	0 to 48	49 to 103
Substance administered	—	Fructose	Fructose
Fructose	0	350	476
Glucose	−39	−105	−83
Pyruvic acid	0.3	−4.6	−16
Lactic acid	16	−49	−63

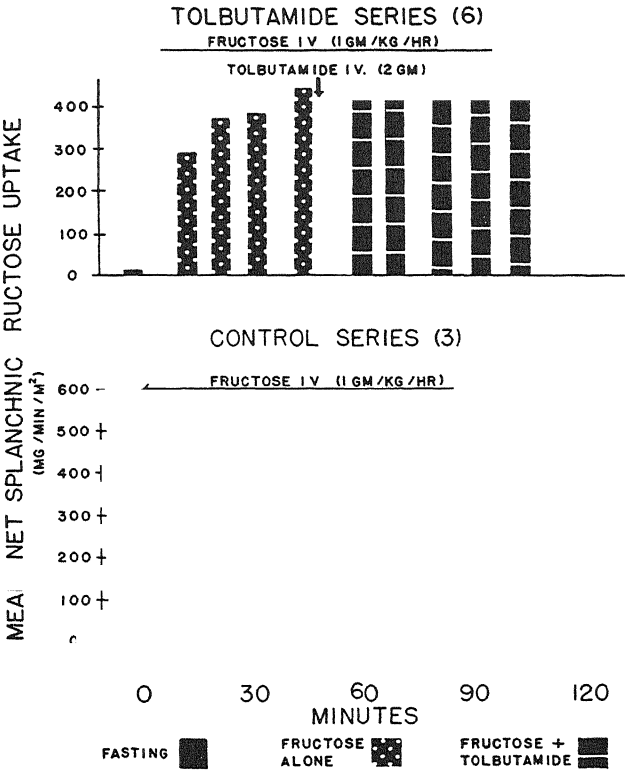

FIGURE 4. The effect of tolbutamide on the mean net splanchnic uptake of fructose during the administration of fructose

of fructose which, during the latter part of the test, was equal to about two fifths of the rate of administration of the hexose.

The mean net splanchnic output of glucose increased when fructose was given (FIGURE 5). Following tolbutamide administration, there was an early decline in the glucose output. In the control series a decline in glucose output was also noted, but the decrease in output appeared to occur somewhat later in the course of fructose administration. It is of interest that the diabetic patient mentioned above who showed no significant hypoglycemic response to a test dose of tolbutamide also failed to have an early decline in net splanchnic glucose output following the administration of the drug. As seen in TABLE 1, the mean rate of splanchnic glucose output during the first part of the test was similar in both the tolbutamide and control series; during the latter half of the test there was a moderate decline in the control series, but a marked decrease after tolbutamide administration.

Fructose administration resulted in a mean net splanchnic output of pyruvic and lactic acids (TABLE 3). The rate of output of these acids increased during the first half of the test and was then maintained during the remainder of the experiment. An effect of tolbutamide on the splanchnic

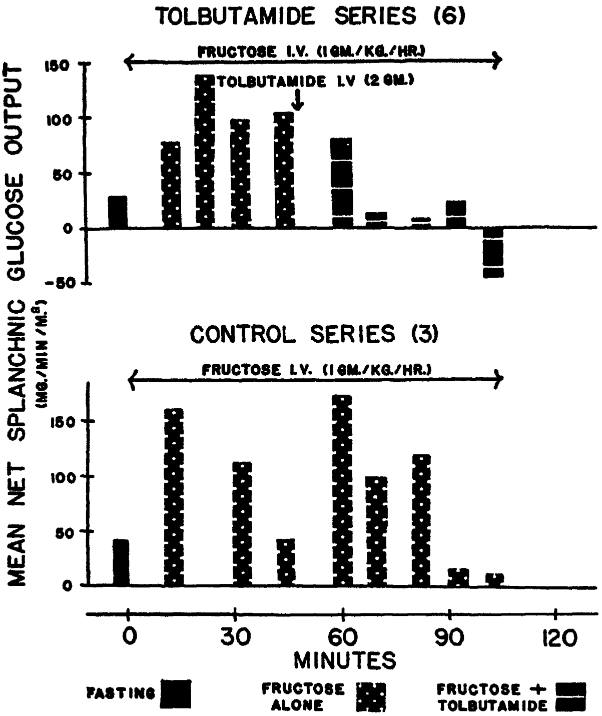

FIGURE 5. The effect of tolbutamide on the mean net splanchnic output of glucose during the administration of fructose.

production of these acids was not demonstrable under the circumstances of these experiments.

Discussion

Although these preliminary results suggest that tolbutamide diminishes the net splanchnic glucose output during fructose administration, the terminal reduction of output in the control cases necessitates further study of this problem. The spontaneous decline in the net splanchnic glucose output in the control series may be a manifestation of the function of the liver in the regulation of the glucose concentration in the peripheral blood.[15, 16] A rise in the peripheral blood glucose concentration accompanied the administration of fructose and may have acted as a stimulus to the hepatic mechanisms that diminish hepatic glucose output. Since this tendency to a spontaneous decline in the net splanchnic glucose output during fructose administration appears to be less in the diabetic than in the nondiabetic subject, the addition of more diabetic patients to the control group may increase the contrast in the results in the tolbutamide and control series. The administration of tolbutamide earlier in the course of fructose infusion might make it possible to demonstrate a more distinct difference as compared to the control cases. This type of experiment does not make it possible to distinguish changes that are due to an effect on the conversion of fructose to glucose in the splanchnic system from those that result from an effect on the release of glucose from the system. A differentiation between a direct action of tolbutamide or an indirect effect mediated through insulin is also not possible from these data. Because of the large net splanchnic output of pyruvic and lactic acids during fructose administration, changes induced by tolbutamide in the metabolism of these acids would probably not be demonstrable unless they were of relatively great magnitude.

Summary

Preliminary results of hepatic vein catheterization studies in diabetic and nondiabetic patients suggest that tolbutamide may reduce the net splanchnic output of glucose during fructose administration. There is no evident effect on the splanchnic balances of fructose or of pyruvic and lactic acids.

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STUDIES ON THE MECHANISM OF TOLBUTAMIDE HYPOGLYCEMIA IN ANIMAL AND HUMAN SUBJECTS*

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Since 1942 there has accumulated an increasingly impressive body of scientific evidence indicating that the sulfonylurea compounds are extremely effective hypoglycemic agents in both human and animal subjects.[1] The potential usefulness of these drugs in the management of patients with diabetes has provided the stimulus for innumerable clinical trials. However, the fundamental question regarding the mechanism of this hypoglycemic action has not yet been answered. It is obvious that a full understanding of this mechanism is of more than academic interest. Although many classes of drugs may produce hypoglycemia, the usefulness of such drugs as therapeutic agents in diabetes must be dependent upon more than a hypoglycemic action. There must be, in addition, an enhancement of the over-all metabolism of carbohydrate. In order to evaluate the potential therapeutic benefits that may be derived from the sulfonylureas, it becomes important, first, to understand the mechanisms by which the hypoglycemia is produced and, second, to measure certain parameters that might reflect alterations in metabolic processes known to be deranged as a consequence of impaired carbohydrate utilization. The experiments to be described were designed to study the action of tolbutamide in animal and human subjects, within the framework of the concepts noted.

Since hypoglycemia may result from a decreased release of glucose from the liver, an increased utilization of glucose in the peripheral tissues, or a combination of the two, an effort was first made to determine the major locus of tolbutamide action. The evidence to be presented supports a hepatic mechanism of action. In order to determine the effect of tolbutamide upon over-all carbohydrate metabolism, studies were made of amino acid incorporation into liver protein, a metabolic system known to be dependent upon adequate utilization of carbohydrate within the liver.[2] An enhancement of the protein "synthetic" mechanisms in liver following tolbutamide therapy was found. These observations suggest that tolbutamide decreases the release of glucose from the liver, while at the same time increasing the over-all glucose utilization by that tissue.

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Studies in Animal Subjects

These experiments were designed to study the effect of tolbutamide upon hepatic glucose release and muscle glucose uptake in rat tissues, isolated at varying times following an intraperitoneal injection of tolbutamide. In this way, a dynamic correlation between the level of blood sugar and the changes in liver and muscle metabolism could be obtained. Male Sprague-Dawley rats weighing 150 to 175 gm. were used and were studied in the fed state. At 0 time, 20 mg. of tolbutamide per 100 gm. body weight were administered intraperitoneally. Groups of rats were sacrificed at 0 time and at 15-min. intervals over the course of one hour. Blood sugars were obtained, and the hemidiaphragms and livers were removed. Liver slices were prepared, and the tissues were immersed in 2.0 ml. of a Krebs-Ringer-phosphate buffer at *p*H 7.4. In the studies of diaphragm metabolism, the phosphate buffer contained 200 mg. per cent of glucose. The tissues were incubated for 1 hour in an atmosphere of oxygen at 37°C. in a Dubnoff shaker, at a shaking rate of 100 cycles per minute. At the end of the experiment the concentrations of glucose[3] and pyruvate[4] in the medium were measured, and the tissues were blotted dry and weighed. The liver slice data were recorded as γ of glucose or pyruvate released per gram of liver per hour. The diaphragm data were recorded as γ of glucose taken up from the medium or γ of pyruvate formed per 100 mg. of muscle per hour.

The data obtained in the liver slice experiments may be seen in TABLE 1. As the blood sugar of the intact animal decreased, a concomitant decrease in the release of glucose from the liver slices was noted. No change in liver glucose-6-phosphatase activity could be found at a time when the hepatic release of glucose was already depressed.[5] In TABLE 2, the changes in glucose uptake of the diaphragms may be seen. Despite the development of hypoglycemia, no increase in glucose uptake occurred. However, a significant decrease in accumulation of pyruvate in the diaphragm incubation medium was noted. In order to determine whether this represented an increased utilization or a decreased formation of pyruvate, diaphragms were incubated in 10^{-3} *M* sodium pyruvate without glucose. No differences in pyruvate

TABLE 1

EFFECT OF INTRAPERITONEAL INJECTION OF TOLBUTAMIDE ON GLUCOSE RELEASE AND PYRUVATE FORMATION OF ISOLATED RAT LIVER SLICES

Time after injection (min.)	No. of expts.	Change from control values		
		Hepatic glucose release γ/gm. liver/hr.	Hepatic pyruvate formation γ/gm. liver/hr.	Blood sugar mg./100 ml.
30	8	−1300	−46	−31
45	8	−2500	−50	−25

TABLE 2
EFFECT OF INTRAPERITONEAL INJECTION OF TOLBUTAMIDE ON GLUCOSE UPTAKE AND PYRUVATE PRODUCTION OF ISOLATED RAT DIAPHRAGM

Time after injection (min.)	Glucose uptake		Pyruvate formation		Blood sugar	
	No of expts.	γ/100 mg./hr. ± S.E.	No. of expts.	γ/100 mg./hr. ± S.E.	No. of expts.	mg./100 ml.
0	20	340 ± 30	24	22 ± 1.3	10	102
30	23	300 ± 23*	24	19 ± 1.0*	10	74
45	20	380 ± 28*	20	17 ± 0.8†	10	81
60	12	400 ± 36*	12	20 ± 1.1*	6	74

* $P > 0.1$ compared to 0 minutes
† $P < 0.01$ compared to 0 minutes
> 0.001

utilization were observed in tolbutamide-pretreated rats. These observations suggest an actual decrease in pyruvate formation in muscle of tolbutamide-treated animals.

Similar experiments, using 0.025 to 0.04 units of insulin intraperitoneally instead of tolbutamide, were performed to determine whether measurements of glucose uptake by isolated diaphragms could be expected to reflect changes during a type of hypoglycemia that is known to be associated with enhanced carbohydrate utilization. TABLE 3 shows the highly significant increases in diaphragm glucose uptake and pyruvate accumulation. It must be pointed out, however, that the insulin hypoglycemia was more intense than that associated with tolbutamide.

TABLE 3
EFFECT OF INTRAPERITONEAL INJECTION OF INSULIN ON GLUCOSE UPTAKE AND PYRUVATE FORMATION OF ISOLATED RAT DIAPHRAGM

Time after injection (min.)	Glucose uptake		Pyruvate formation		Blood sugar	
	No. of expts.	γ/100 mg./hr. ± S.E.	No. of expts.	γ/100 mg./hr. ± S.E.	No. of expts.	mg./100 ml.
0	6	320 ± 17	6	19 ± 1.1	3	107
30	10	460 ± 34*	10	22 ± 1.8‡	5	48
45	6	540 ± 43†	6	26 ± 2.0*	3	50
60	6	500 ± 29†	6	26 ± 2.5§	3	56

P compared to 0 minutes
* $P < 0.01$
† $P < 0.001$
‡ $P > 0.1$
§ $P < 0.05$

These studies in rats indicate that following tolbutamide, a decrease in hepatic glucose release occurs concomitant with the development of hypoglycemia. No evidence of enhanced peripheral utilization of carbohydrate was obtained. Peripheral formation of pyruvate in tissue appeared to decrease.

Studies in Human Subjects

Hepatic glucose output. To investigate the effect of tolbutamide upon hepatic glucose output in human subjects, hepatic vein catheterization studies were performed in fasted patients given 1.0 gm. of tolbutamide intravenously. Hepatic blood flow was determined by the bromsulphalein (BSP) extraction method of Bradley *et al.*[6] Arterial blood was obtained from an indwelling femoral artery needle. Glucose analyses were made on simultaneously obtained hepatic vein and femoral arterial samples.

A marked decrease in hepatic venous-arterial (V-A) glucose difference was noted following tolbutamide injection in 4 subjects, 2 of whom were normal and 2 of whom were cirrhotic (TABLE 4). In another subject, no drop in hepatic V-A glucose difference was observed; this subject was very agitated during the procedure, but did develop some hypoglycemia. A sixth subject, suffering from diabetes following subtotal pancreatectomy, showed no narrowing of the hepatic V-A glucose difference and manifested little hypoglycemia. Since the calculation of the hepatic glucose output is dependent upon the product of the hepatic V-A glucose difference and the estimated hepatic blood flow (EHBF), it was necessary to determine the effect of tolbutamide upon EHBF. This was of importance also, since a decrease in EHBF in dogs following tolbutamide administration had previously been reported.[7] In 7 patients (TABLE 5), no significant change in EHBF was noted for 45 minutes following injection; however, at 1 hour and later, following tolbutamide, a rise in EHBF was noted. This delayed rise, similar to that following insulin administration,[8] has been attributed to the release of epinephrine that accompanies recovery from hypoglycemia. Due to tech-

TABLE 4
EFFECT OF TOLBUTAMIDE ON HEPATIC V-A GLUCOSE DIFFERENCE (mg. %)

Experiment	1	2	3	4
Control (mean of 3 periods)	4.0 ± 0	9.3 ± 2.6	5.5 ± 3.3	10.6 ± 4.4
Time after 1.0 gm. tolbutamide I.V. (min.)				
15	4.4	0.8	9.6	2.2
30	2.0	−1.8	1.6	9.9
45	0.8	2.8	10.2	0.6
60	6.0	1.2	13.0	12.0
75	6.6	3.6	9.6	17.8
90		9.4	8.6	

TABLE 5
EFFECT OF TOLBUTAMIDE ON ESTIMATED HEPATIC BLOOD FLOW (cc./min.)
Mean of 7 experiments

Control periods (1	1410)	
2	1510	1430
(3	1360)	
Time after tolbutamide 1.0 gm. I.V. (min.)		
15.	1430	
30	1440	
45	1370	
60	1730	
75	1750	
90	2210	

nical difficulties, calculation of the true hepatic glucose output was possible in only 2 patients, who showed a drop from 65 to 16 mg. glucose output per min. at 30 min. after tolbutamide and from 172 to 37 and 10 mg. per min. at 15 and 45 min. respectively.

The narrowing of the hepatic V-A glucose difference and the decrease in hepatic glucose output noted in these experiments is similar to those reported following insulin administration,[8] but occurred somewhat more slowly. Evidence of decreased hepatic glucose output after tolbutamide administration in dogs[9] and in human subjects[10] has previously been reported. Kibler and Gordon[10] noted, however, that several of their patients developed hypoglycemia without a drop in hepatic glucose output. It is possible that the narrowing of the hepatic V-A glucose difference that follows the injection of tolbutamide is so transient that it is missed because of too infrequent sampling.

Peripheral glucose utilization. Peripheral A-V glucose differences were measured following intravenous administration of 1.0 gm. of tolbutamide to fasted human subjects. Samples were obtained from indwelling needles in the antecubital vein and femoral artery. In no case was any increase in peripheral A-V glucose difference observed (FIGURE 1). Other investigators, using glucose infusion techniques, also have been unable to demonstrate increased peripheral utilization of glucose after tolbutamide,[7] in contrast to the peripheral effect of insulin. The only report of an increase in peripheral A-V glucose difference following tolbutamide injection[11] was obtained in experiments using capillary "arterial" instead of true arterial blood sugars. This observation is of interest since it is apparently more difficult to demonstrate an increase in peripheral A-V glucose difference following the injection of small doses of insulin when true arterial blood sugars are obtained[12] than when one uses capillary "arterial" values.[13] Furthermore, in a small number of our experiments in which A-V glucose differences were measured after 3 units of insulin intravenously, no widening of A-V glucose was obtained. However, small doses (1 unit) of insulin given intra-arterially produced

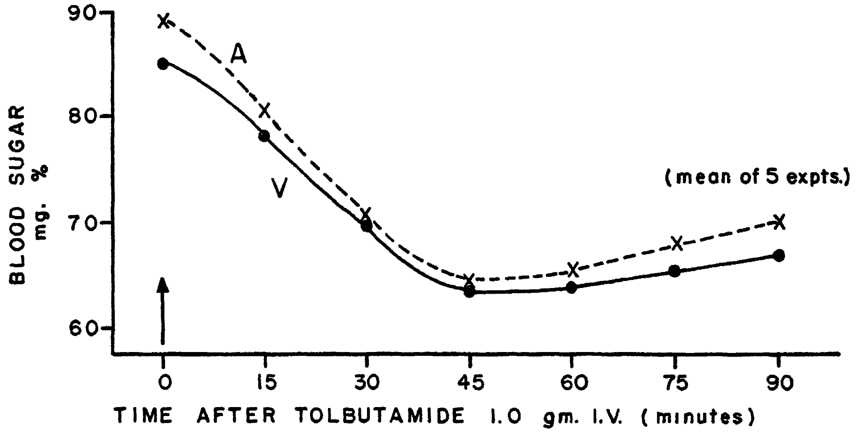

FIGURE 1. Effect of tolbutamide on peripheral A-V glucose difference.

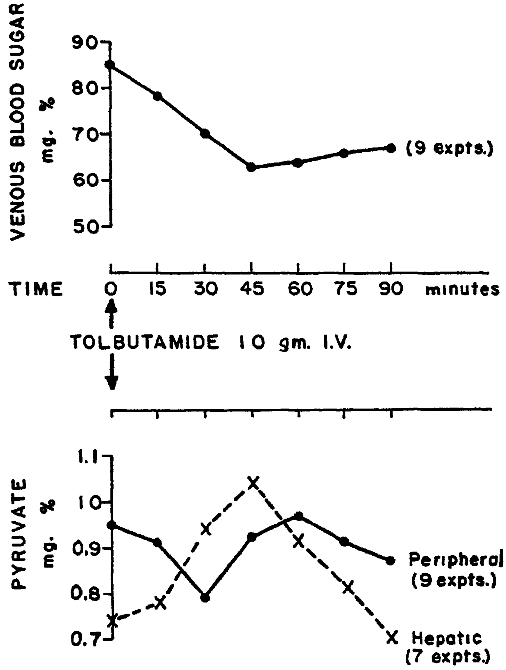

FIGURE 2. Effect of tolbutamide on hepatic and peripheral venous pyruvate levels.

prompt widening of the A-V difference across the injected limb,[12] indicating increased muscle uptake. In preliminary experiments we have been able to confirm this finding, but have been unable to demonstrate increased glucose uptake following intra-arterial injection of equivalent doses of tolbutamide.

It may be concluded from these experiments that tolbutamide, in contrast to insulin, produces no direct effect upon muscle glucose uptake. In addition, no evidence of an indirect effect on peripheral utilization of carbohydrate was demonstrated following tolbutamide. It is noteworthy, however, that it is difficult to demonstrate a peripheral effect of small doses of intravenously administered insulin.

Pyruvate studies—hepatic and peripheral. Following 1.0 gm. of tolbutamide intravenously, peripheral and hepatic venous levels of pyruvate were measured over the course of 1 hr. Peripheral pyruvate blood levels manifested an initial fall (FIGURE 2) similar to that previously reported by Hennes *et al.*[14] However, in contrast to this peripheral effect, a significant rise in hepatic vein pyruvate levels was observed (FIGURE 2). This increase averaged 41 per cent and it correlated in time with the point of maximal narrowing of hepatic V-A glucose difference. Hepatic vein pyruvate levels did not rise in patients who showed no decrease in hepatic glucose output. Although the rise in pyruvate was equimolar to only one twentieth of the drop in hepatic glucose output, it would appear that a portion of the glucose retained by the liver was converted to pyruvate and presumably made available for complete oxidation.

The foregoing data suggest that tolbutamide hypoglycemia in human subjects resulted from a retention of carbohydrate by liver with an associated increase in hepatic glucose utilization as evidenced by the rise in hepatic pyruvate.

Tolbutamide and Hepatic Protein Metabolism

In an effort to obtain evidence bearing on the problem of the effect of tolbutamide on the over-all metabolism of carbohydrate in the liver, studies of hepatic protein "synthesis" were made. It has been shown that the incorporation of C^{14}-glycine into peptides and protein of liver slices is dependent upon the availability of the intermediates of glycogen and glucose degradation. Fasting, which may be described as exogenous carbohydrate deprivation, and diabetes, as endogenous carbohydrate deprivation, both significantly depress this system.[2] Restoration of glycine incorporation occurs with refeeding, or, in the latter condition, with administration of glucose and insulin.

To test the effect of tolbutamide on this system, rats were pretreated for 4 to 5 days with oral administration of the drug. FIGURE 3 shows the experimental conditions utilized. Both the control and experimental animals were fasted for periods of approximately 24 to 48 hours. They were then sacrificed; their livers were removed, and the *in vitro* incorporation of C^{14}-glycine into the liver-slice protein was studied by the methods previously described.[2] The rate of incorporation, or specific activity (S.A.), was expressed as counts/min. of $C^{14}/100\ \gamma$ liver-slice protein. It may be seen from FIGURE 3 that one intraperitoneal injection of the drug had no effect on the S.A. of liver protein. Moreover, as one would expect, with increased periods of fasting the S.A. of the controls fell markedly, while in all of the tolbutamide-treated animals the S.A. was maintained at a higher level.

TABLE 6
EFFECT OF TOLBUTAMIDE ON *In Vitro* INCORPORATION OF C^{14}-GLYCINE INTO LIVER-SLICE PROTEIN

	No expts	Specific activity ± S.E. counts/min /100 γ protein
Control rats	24	65 ± 6.2
Tolbutamide-treated rats	23	101 ± 7.0*

* $P < 0.001$

TABLE 6 indicates the striking increase in S.A. noted in the tolbutamide-pretreated rats as compared with controls.

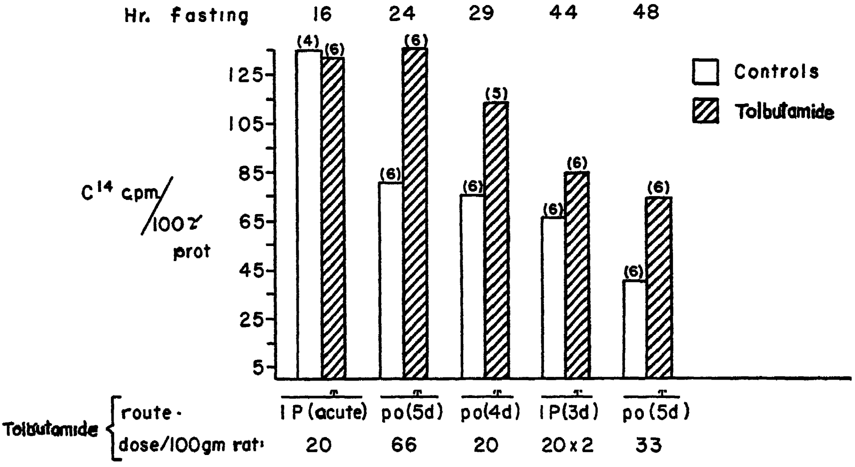

FIGURE 3. Effect of tolbutamide on the *in vitro* incorporation of C^{14}-glycine into rat liver protein

This finding indicates that tolbutamide enhances protein "synthesis" in the fasted animal. Since, as noted, this system is dependent upon an active glycolytic mechanism, the present study would indicate that tolbutamide, while retaining carbohydrate in the liver, actually increases its over-all utilization by that tissue.

Summary

Within the framework of the experiments described, it appears that tolbutamide is an effective hypoglycemic agent. The gross mechanism of this hypoglycemia appears to be hepatic; more specifically, it appears to operate by effecting a decrease in the hepatic glucose output. Associated with this effect, there is increased utilization of carbohydrate by the liver, as evidenced by increased hepatic vein pyruvate and a striking enhancement of amino acid incorporation into liver-slice protein.

Although no increased utilization of carbohydrate in the peripheral tissues could be demonstrated, it cannot be concluded that this process does not occur. It is of some interest, however, to note that peripheral formation of pyruvate is decreased in the absence of significant change in glucose uptake.

The primary mechanism of tolbutamide action is under study. At the present time it may best be described as consistent with a hepatic action that simulates the insulin response in every way.

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STUDIES ON THE SITE OF ACTION OF THE ARYLSULFONYLUREAS IN MAN. II.*

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Departments of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, and the Baker Clinic Research Laboratory, New England Deaconess Hospital, all in Boston, Mass.

Previous studies from this laboratory have been concerned with the characterization of arylsulfonylurea-induced hypoglycemia as due primarily either to increased peripheral glucose utilization or to decreased hepatic glucose synthesis or release.[1] It was observed that the hyperglycemia that follows the intravenous administration of fructose and galactose loads to patients with diabetes mellitus was considerably decreased by the previous administration of carbutamide or tolbutamide, although glucose tolerance remained unchanged. Since the liver is principally responsible for the conversion of fructose and galactose to glucose, this observation was interpreted as suggesting an effect of the hypoglycemic sulfonylureas upon hepatic glucose synthesis or release. This interpretation is in accord with the findings of others in isolated tissues,[2, 3] in experimental animals,[4, 5] and in man.[6, 7, 8, 9] It is appreciated that these studies in no way excluded other sites of action of these compounds.

In these earlier studies an effect of the hypoglycemic sulfonylureas upon peripheral glucose utilization in man was sought, but not found.[1] Since an important hypothesis concerning the mode of action of these compounds is that of increased insulin synthesis or release,[10, 11] a physiological event that should lead to increased peripheral glucose utilization, re-evaluation of some metabolic indices frequently associated with increased peripheral glucose utilization has been undertaken. In addition, an attempt has been made to demonstrate the postulated increased insulin secretion by measuring insulinlike activity in plasma before and after intravenous administration of the compound.

Material and Methods

All studies were carried out on the metabolic ward of the Peter Bent Brigham Hospital. Young male volunteers in good health served as normal subjects, each subject serving as his own control. Plasma insulinlike activity was assayed by a slight modification of the method of Vallance-Owen,[12] using glucose uptake by the rat hemidiaphragm as an indicator of insulinlike activity. Each assay was carried out in duplicate and, in each instance, 3 standard insulin concentrations were simultaneously carried through the

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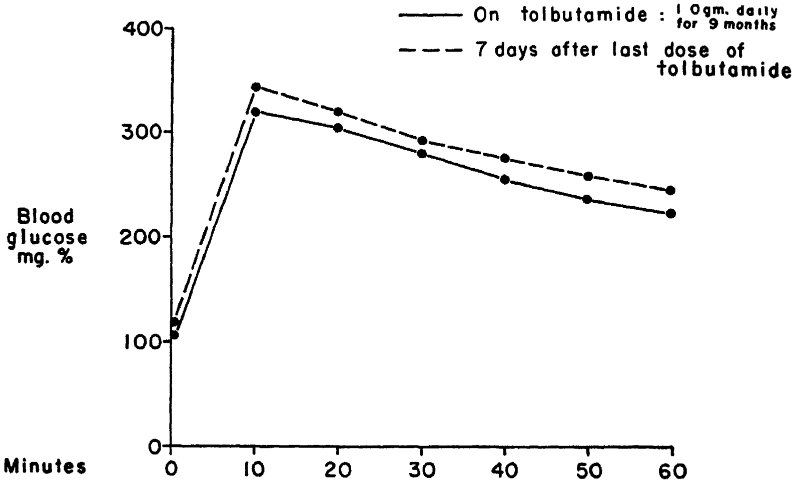

FIGURE 1. Glucose tolerance tests in a previously hypophysectomized patient (M.G., ♂, 46, 5G484) with diabetes mellitus, maintained on cortisone (37.5 mg./24 hr.) and thyroid (60 mg./24 hr.). Glucose (0.5 gm./kg.) infused intravenously between 0 and 10 minutes.

assay procedure, each in duplicate. Only values giving insulinlike activities equivalent to insulin concentrations lying between 2 simultaneously assayed standard concentrations were used. In our hands, duplicate values obtained with this assay procedure have agreed within 5 to 10 parts in 100 within the range of 100 to 10,000 microunits per ml. of plasma, while the significance of values below 100 microunits per ml. has appeared doubtful. Hence, activities corresponding to insulin concentrations below 100 microunits per ml. have been expressed as "100 microunits per ml. or less." Blood samples for insulin assay were collected with heparin, and plasma was separated immediately by centrifugation, stored at 4° C., and assayed within 48 hours. When respiratory quotients were measured, samples were collected in Douglas bags and analyzed for carbon dioxide with a Liston-Becker infrared spectrophotometer and for oxygen with a Pauling oxygen meter. Other methods used were as previously described.[1]

Results

The results obtained by measuring variations of certain indices of peripheral glucose utilization are summarized in TABLE 1. The glucose tolerance tests

TABLE 1

MEASURED INDICES OF "PERIPHERAL GLUCOSE UTILIZATION"

No demonstrable tolbutamide effect on
  Intravenous glucose tolerance
  Serum phosphate levels after glucose I.V.
  Blood pyruvate levels after glucose I.V.
  Respiratory quotient
  Nitrogen balance
Probable tolbutamide effect on
  Blood ketones
  Plasma unesterified fatty acids

with simultaneous determination of changes in blood pyruvate and serum phosphate levels were carried out, as a rule, in diabetic patients before and several days after the beginning of oral tolbutamide (Orinase) therapy. The study shown in FIGURE 1, however, was carried out after a period of 9 months on oral tolbutamide, 1.0 gm. daily, and again 7 days after the last dose of the compound. This patient had been hypophysectomized 11 months previously and his initial response to tolbutamide has been previously recorded.[1] The profile of the glucose tolerance curve was not significantly affected either by the institution of tolbutamide therapy or by its withdrawal.

FIGURE 2 demonstrates that the respiratory quotient of a patient with untreated diabetes mellitus failed to increase after acute tolbutamide administration by mouth, despite a marked hypoglycemic response. In this study, preliminary observations had been made in order to obtain closely similar blood glucose curves after tolbutamide and insulin, with regard to both total

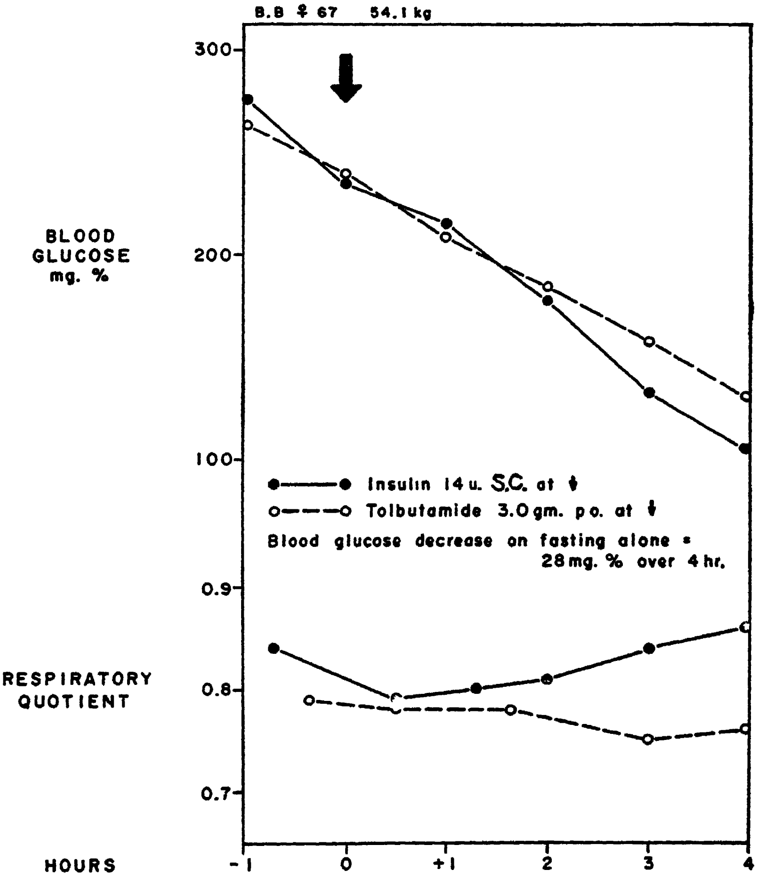

FIGURE 2. The effect of tolbutamide on blood glucose levels and on respiratory quotient in a patient with diabetes mellitus.

decrease in blood glucose and rate of fall. The subcutaneous administration of 14 units of crystalline insulin approximated the effect of tolbutamide on blood glucose, and resulted in a small but probably significant rise in respiratory quotient.

The effect of tolbutamide on blood ketone and plasma unesterified fatty acid levels are listed in TABLE 1 as probable effects since they have been obtained thus far in only a small group of patients. In these patients the oral administration of single doses of tolbutamide, 2 to 4 gm., resulted in decreased levels of blood ketones or plasma unesterified fatty acids within 4 hours in patients who also showed a blood glucose response. Further studies of this phenomenon are under way.

Plasma levels of insulinlike activity in normal subjects are shown in FIGURES 3 and 4, as are the corresponding blood glucose levels. In the studies

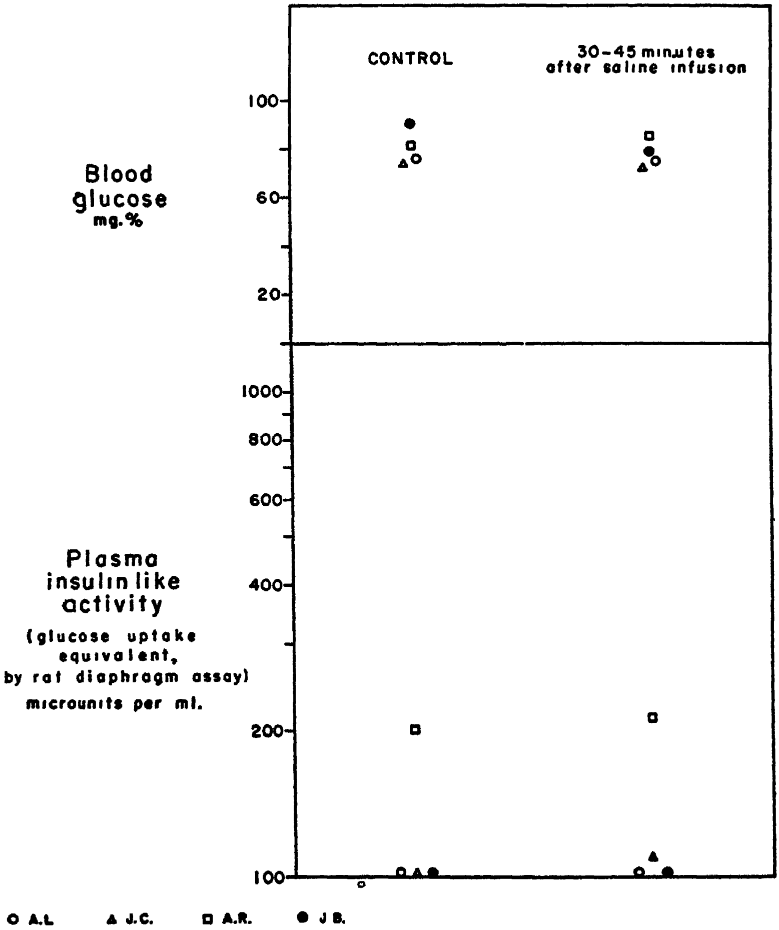

FIGURE 3. Plasma insulinlike activity in fasted normal subjects.

described in FIGURE 3, blood samples were obtained before and 30 to 45 minutes after a rapid saline infusion (subjects A. L. and J. C.) or simply after continued fasting (subjects A. R. and J. B.). In the studies described in FIGURE 4, blood sampling was carried out before and 10 to 40 min. after the rapid (5 min. or less) intravenous infusion of tolbutamide (37.5 mg. per kg.) dissolved in saline. Tolbutamide administration resulted in marked hypoglycemia, and it should be emphasized that the blood glucose level decreased by about 50 per cent (or by about 40 mg. per cent) within 10 min. In no instance did plasma insulinlike activity increase after tolbutamide administration. The timing of the blood samples obtained will be discussed later in this paper.

FIGURE 5 shows blood glucose and plasma insulinlike activity levels in two

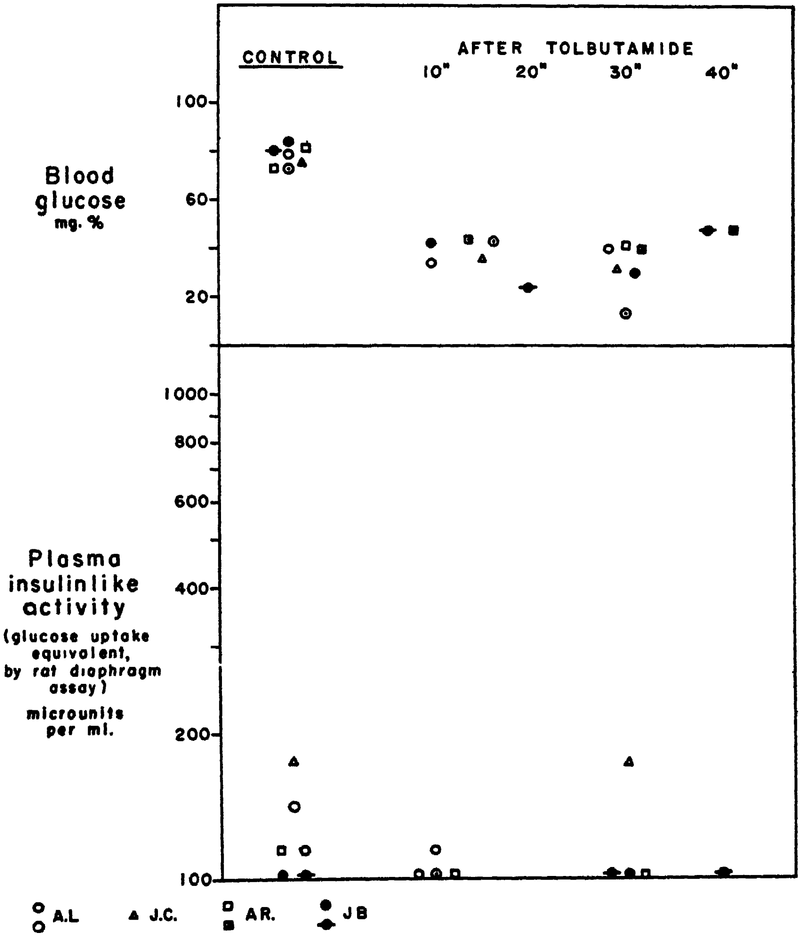

FIGURE 4 Plasma insulinlike activity in normal subjects after intravenous tolbutamide (37.5 mg kg).

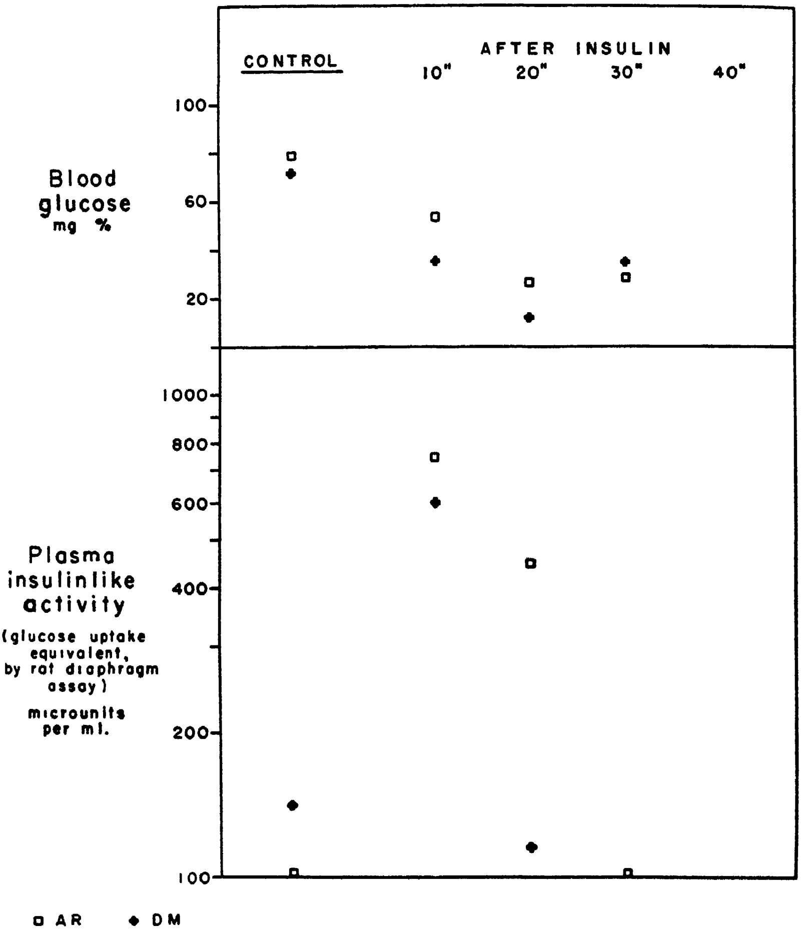

FIGURE 5. Plasma insulinlike activity in normal subjects after intravenous insulin (0.1 unit/kg.).

normal subjects, before and 10 to 30 min. after the intravenous administration of glucagon-free insulin (0.1 unit per kg.). In both instances, plasma insulinlike activity was markedly elevated 10 min. after the insulin injection, and this elevation persisted in one of the two subjects 10 min. later. The blood glucose changes after insulin were quite similar to those obtained with tolbutamide; in particular, blood glucose decreased by about 50 per cent, or 40 mg. per cent, within 10 min.

A final and as yet preliminary observation is shown in FIGURE 6. A metabolically normal subject was given a constant glucose infusion (0.5 gm. per kg. per hour) over 8 hours, using a Bowman constant infusion pump. Blood glucose levels first increased to 180 mg. per cent at one hour, then returned almost to fasting levels and remained close to fasting levels throughout the

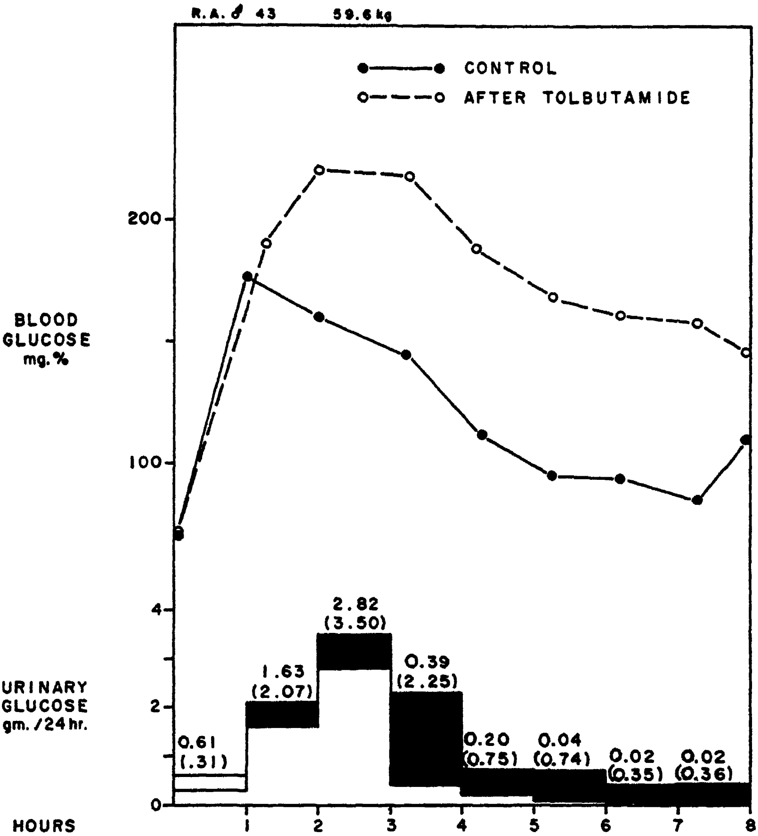

FIGURE 6. Constant intravenous glucose infusion (0.5 gm./kg./hr.) before and after tolbutamide (4.0 gm. q. d. × 8) in a metabolically normal subject. Dietary intake during tolbutamide administration: 330 gm. carbohydrate; 67 gm. protein; 93 gm. fat.

latter part of the infusion. This represents a typical normal response, commonly interpreted as the result of increasing insulin secretion in response to the continuous infusion of glucose. The procedure was then repeated after 8 days of tolbutamide therapy, 4 gm. daily administered in two doses by mouth. The last dose was given 14 hours before initiating the second glucose infusion. Throughout the period of tolbutamide therapy, the subject was maintained on a constant dietary regimen consisting of 300 gm. carbohydrate, 67 gm. protein, and 93 gm. fat. This diet exceeded the subject's customary dietary carbohydrate intake by approximately 100 gm. daily, while the protein and fat approximated his usual requirements. It was hoped that the additional carbohydrate might minimize any tendency to hypoglycemic episodes; and, indeed, no symptomatic hypoglycemic episodes occurred. Fasting blood glucose levels, however, were somewhat lower than in the control period. It is apparent from the data shown in FIGURE 6 that glucose tolerance, as measured by this rather severe stress, was strikingly decreased after

8 days on tolbutamide at the relatively high dose level of 4 gm. daily. It is premature to speculate on this finding; the observation is presented now purely for more general consideration of a rather striking effect

Discussion

If the hypoglycemic arylsulfonylureas exert their action chiefly by stimulating insulin secretion or by potentiating insulin effectiveness, the hypoglycemia thus produced should be accompanied by the metabolic alterations usually associated with insulin hypoglycemia. Among the changes that accompany the hypoglycemia produced by insulin, those concerning indices of peripheral glucose utilization are perhaps the most characteristic. It is evident from the summary presented in TABLE 1 that tolbutamide-induced hypoglycemia was not accompanied in these studies by clear-cut evidence of increased peripheral glucose utilization. However, it should be clearly understood that all available indices of peripheral glucose utilization in the intact human organism are both nonspecific and relatively insensitive. When attempts are made to approximate the hypoglycemic effect of tolbutamide with appropriate amounts of insulin, the evidence for an insulin-induced increase in peripheral glucose utilization quite often *also* lacks conclusiveness, as illustrated in FIGURE 2. These studies are therefore interpreted at present as failing to support the hypothesis of increased insulin secretion or effectiveness as a major component of tolbutamide-induced hypoglycemia, but by no means as ruling out this mechanism of action.

The interpretation of the measurements of plasma insulinlike activity before and after intravenous tolbutamide is equally difficult. The particular experimental design was selected with the following observations in mind: (1) the most sudden changes in blood glucose are obtained in normal subjects given relatively large doses of tolbutamide by the intravenous route; (2) Goetz[13] has reported a maximal increase in arteriovenous glucose difference approximately twenty minutes following the intravenous infusion of tolbutamide; (3) the decrease in blood glucose obtained under these conditions compares favorably with the changes observed during standard intravenous insulin tests; (4) it has been stated[14] that the early hypoglycemic period that follows the administration of hypoglycemic arylsulfonylurea is the period most likely to represent stimulation of insulin secretion; (5) it may be assumed that normal subjects dispose of an adequate insulin reserve. The conditions selected appeared, therefore, to provide optimum conditions for the demonstration of increased insulin secretion, if such occurred.

Plasma insulinlike activity did not increase within 10 to 40 minutes following tolbutamide administration to 4 normal subjects in the course of 7 separate experiments, thus again failing to provide evidence for increased insulin secretion after tolbutamide administration. It is fully appreciated that this negative result is of limited significance only. The measurement used lacks specificity ("insulinlike activity" rather than insulin), sensitivity, and precision. However, it should be pointed out that when glucagon-free insulin was given intravenously in amounts producing a similar degree of hypoglycemia at a similar rate, definitely elevated levels of plasma insulinlike activity were obtained within 10 to 20 minutes of the administration of the hormone (FIGURE 5). Furthermore, elevated levels could also be demonstrated with ease during and following glucose infusions.

In conclusion, the studies carried out in this laboratory have *as yet* failed to provide evidence suggesting the increased peripheral utilization of glucose or the increased secretion of insulin in *man* as a result of tolbutamide action. This is in agreement with most studies in man that have been reported from other laboratories, although Goetz[13] has described increased arteriovenous glucose differences after administration of the drug. Whereas in *animals* the evidence suggesting increased secretion of insulin after tolbutamide[14, 15] is quite convincing, in *man* the major argument in favor of a tolbutamide-induced insulin secretion resides in the observation that pancreatectomized patients and juvenile diabetics fail to respond to sulfonylurea administration. It is important to keep in mind, however, that the tissues of pancreatectomized organisms undergo a multitude of metabolic rearrangements and adaptive changes, including major alterations of glycogen deposition and synthesis, of glucose utilization and production, and of anabolic and catabolic reactions concerned with the metabolism of proteins and fats. Many of these changes may persist for as long as 24 to 48 hours after the administration of insulin.[16] The abnormal response of these profoundly modified tissues to a given agent does not imply that the pancreas is directly involved in the mechanism of action of this agent. Although an effect of the sulfonylureas on insulin secretion or effectiveness in *man* has not been ruled out, present evidence suggesting a direct effect of these compounds on hepatic glucose synthesis or release is stronger and more convincing to the authors. A complex effect combining peripheral and hepatic actions, and possibly still other activities, represents a distinct possibility at this time.

Finally, it should be understood that the finding of a major effect of the arylsulfonylureas upon hepatic glucose synthesis or release does not imply that the hypoglycemia thus produced in diabetic patients is unfavorable or even harmful. In the presence of the increased hepatic gluconeogenesis characteristic of diabetes mellitus, a decreased release, as glucose, of the carbohydrate formed, resulting perhaps in an increased hepatic retention of carbohydrate, as well as in an increased release of other intermediates such as lactate and pyruvate, might well be a favorable effect. Whether this is indeed the case remains, of course, to be established.

Summary

(1) Further studies of the hypoglycemic effect of tolbutamide have been carried out in man in an attempt to characterize the tolbutamide-induced hypoglycemia as the result of either increased peripheral utilization of glucose or decreased hepatic release of glucose. The changes observed suggest that increased peripheral glucose utilization is not a major component of tolbutamide action.

(2) Plasma insulinlike activity was estimated by the rat-diaphragm assay technique before and after the intravenous administration of tolbutamide to

healthy young males. Although marked hypoglycemia resulted, no elevation of plasma insulinlike activity could be detected.

(3) In the authors' opinion, the effect of tolbutamide on hepatic glucose release or synthesis is at present the best documented effect of the compound in man.

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A CONCEPT OF THE MECHANISM OF SULFONYLUREA-INDUCED HYPOGLYCEMIA BASED ON STUDIES OF GLUCOSE AND PENTOSE DISPOSITION IN MAN

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The precise mode of action of the sulfonylurea compounds is understood neither in laboratory animals nor in humans. Functioning pancreatic β cells appear to be a prerequisite for their hypoglycemic effect. Diabetics classified as of the "juvenile type," a designation reserved for a group of diabetics believed to be completely insulin-deficient, do not respond to the sulfonylureas, and the hypoglycemic effect of exogenous insulin is not consistently enhanced by these compounds. Two principal lines of approach have been and are being pursued to explain the hypoglycemic action of the sulfonylureas. The first deals with the presence of more effective endogenous insulin. This might be brought about by increasing the rate of formation or liberation of insulin, by inhibiting its destruction or excretion, or by a synergistic or potentiating action. The second proposes that the sulfonylureas produce a hepatic effect, such that one or more of the enzymatic processes essential to the formation and release of glucose are inhibited. Evidence for the latter possibility is suggested by the fact that increased hepatic glycogen stores are found after the administration of sulfonylureas. The accumulated information covering these controversial matters can be found in many publications dealing with the sulfonylureas and will not be repeated here.[1-4]

The studies to be presented were undertaken to elucidate the mechanism of the hypoglycemic action of these compounds. In view of the wealth of available information regarding the effects of insulin, the first goal was to devise suitable techniques of study with tolbutamide that would approximate closely those employed in previous and fairly well-standardized insulin studies. The availability of the sodium salt of tolbutamide (Orinase Sodium) made it possible to give this substance in rapid single doses intravenously and to compare the effects with those of insulin administered in an identical manner. A variety of studies of glucose metabolism were performed before and after the intravenous administration of insulin and tolbutamide, and changes in blood glucose, phosphate, and pyruvate were measured. The pentoses D-xylose and L-arabinose were administered intravenously, and their disappearance was measured after insulin and sodium tolbutamide. These particular pentoses were selected since Levine and his co-workers[5] have shown that their distribution in eviscerated nephrectomized animals is influenced by insulin; hence, the term "insulin-responsive pentoses" has been suggested for these sugars. More recently these same pentoses have been found to be insulin-responsive in man by Segal, Wyngaarden, and Foley.[6]

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Information derived from such studies in man has suggested a convenient template for comparing the mechanism of the hypoglycemic action of sulfonylureas with that of insulin. Utilizing these particular methods of study, there has evolved the concept that sulfonylureas alter the sensitivity of the liver to endogenous insulin without producing a comparable effect on its peripheral action.

Methods

All studies were carried out in normal male and female subjects ranging in age from 18 to 21 years. Each individual was maintained on a high carbohydrate diet. Prior to each study the subjects were fasted for 14 to 18 hr. and placed at complete rest for approximately 10 hr. An indwelling plastic needle in one arm was utilized as a means of providing frequent and reliable blood samples. The opposite arm was used to administer any other materials required in the particular test being performed. Smoking was not permitted during the test, and nothing was permitted by mouth but water.

The insulin tolerance test was performed according to standard procedure, using 0.1 unit of crystalline insulin per kg. body weight.[7] The glucose-insulin tolerance test described by Fraser, Albright, and Smith,[7] and the insulin-glucose tolerance test of Engel and Scott[8] were employed. The rapid intravenous glucose tolerance test of Conard *et al.*[9] was used to measure the rate of blood glucose disappearance.

Blood glucose was measured according to the method of Nelson;[10] blood pyruvate by the Friedemann-Haugen method.[11] Plasma phosphate was determined by the Fiske and SubbaRow method.[12] Plasma levels of sodium tolbutamide were measured using the method of Miller and his co-workers[13] with modifications suggested by Forist and Miller and their associates.[14] The method consists of a chloroform extraction of buffered plasma (*p*H 4.5 to 4.8), concentration of the chloroform extract to dryness, solution of the resulting residue in 95 per cent ethanol, treatment of this solution with Darco G-60, and measurement of the absorbance of the resulting filtrate at 228 mμ. Blood pentose values were determined by the orcinol technique, using blood filtrates in which glucose had been destroyed by treatment with the enzyme glucose oxidase.[15] Glucose was determined by the Nelson modification of the Somogyi method,[10] using the difference between values obtained before and after incubation with the enzyme.

For *in vitro* studies rabbit liver slices, about 0.4 to 0.5 mm. thick, were cut from livers of fed animals. They were incubated at 37° C. for 45 min. in 0.12 *M* NaCl and 0.02 *M* potassium phosphate (*p*H 7.4) in a Warburg apparatus with air as the gas phase. Sodium tolbutamide was dissolved in saline-phosphate buffer for these studies.

Materials

Sodium tolbutamide (Orinase Sodium) was supplied as a sterile powder by The Upjohn Company, Kalamazoo, Mich. The material was put in solution with isotonic sodium chloride to a final concentration of 10 per cent with *p*H 9.12. The solution was clear and colorless. The material was injected intravenously over a 2- to 3-min. interval. Greater concentrations or more rapid injection caused local burning and mild systemic effects. Only crystalline insulin (Merck Sharp & Dohme, Philadelphia, Pa.) was used in studies requiring insulin.

The pentoses D-xylose and L-arabinose used in these studies were obtained from the Pfanstehl Co., Waukegan, Ill. These materials were autoclaved as 10 per cent solutions in distilled water and were found to be sterile and pyrogen-free prior to use.

Results

Hypoglycemia induced with intravenous sodium tolbutamide. The rapid single intravenous administration of sodium tolbutamide (13 to 40 mg. per kg. body weight) is followed by a prompt reduction in blood glucose that appears within 10 min. after its administration (FIGURE 1). This

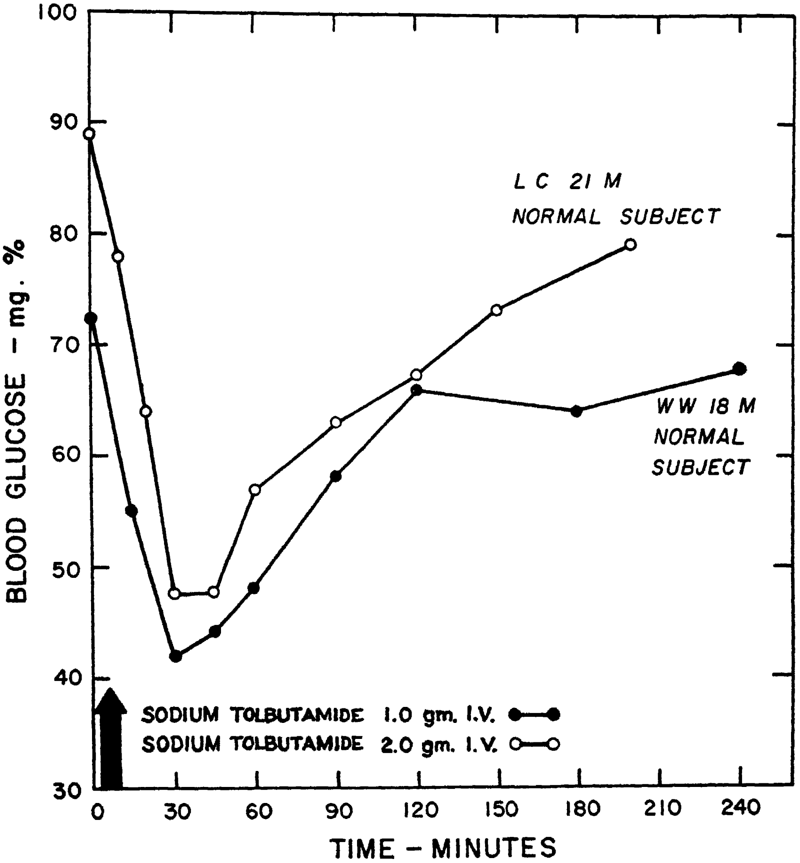

FIGURE 1. The onset of hypoglycemia after intravenous tolbutamide is prompt. The adaptive responses to the hypoglycemia occur rapidly, but there is some delay in the return of the blood sugar to control levels.

depression continues over the ensuing 20 min., reaching its nadir at 30 min. and resembling in a temporal fashion the hypoglycemic response to intravenous insulin. Occasionally the maximum effect is not reached until an additional 10 to 15 min. have elapsed. The hypoglycemia is followed by a gradual return of blood glucose to normal, presumably as adaptive responses develop. However, the over-all glucose response to intravenous tolbutamide differs from that seen with comparable doses of intravenous insulin. With intravenous insulin (0.1 unit per kg. body weight) there is a characteristic return in blood glucose to control values within 90 to 120 min. With comparable doses of intravenous tolbutamide there is a significant delay in the expected return of blood glucose to control values that may persist up to 210 min., with an average of about 180 min. One possible explanation for this delay is the suggestion of Mirsky[16] that this represents a prolongation of the effect of circulating insulin. Evidence from the present studies suggests and supports the alternate possibility of depression of hepatic glycogenolysis occasioned by the presence of tolbutamide in the concentrations employed.

It has been found that the hypoglycemic response in these normal subjects tends to vary directly with the dosage of tolbutamide. Various amounts of tolbutamide (13 to 40 mg. per kg. body weight) were administered intravenously in order to determine the dose that would produce the same hypoglycemic response obtained with a standardized dose of intravenous insulin in a given individual. While 0.1 unit of insulin per kg. body weight intravenously will produce in a majority of subjects a characteristic and reproducible degree of blood glucose depression (40 to 60 per cent), no single dose of tolbutamide that has similar properties has been found. Studies are in progress on this matter, but are hampered by the lack of adequate knowledge regarding the toxicity of tolbutamide in larger acute intravenous doses than those already discussed. It is likely that a standardized hypoglycemic dose of tolbutamide will be found eventually.

In FIGURE 2 the blood glucose response to intravenous tolbutamide in 6 normal subjects is shown. In most instances the degree of blood sugar depression varied directly with dosage of tolbutamide. As noted before, however, in some subjects a point was reached when successive increments in tolbutamide did not result in an increased degree of hypoglycemia. Preliminary studies do not suggest the development of tachyphylaxis with tolbutamide. Therefore, once the intravenous dosage of sodium tolbutamide that produced a 40 per cent or greater lowering of the blood glucose in 30 min. was determined, this amount was used in all subsequent studies in the individual concerned. The importance of determining the proper dosage for each individual must be emphasized. In our experience dosages of intravenous tolbutamide of less than 20 mg. per kg. body weight are not likely to produce consistently a hypoglycemia comparable to intravenous insulin.

In studies in which the blood drug level was determined, there was a prompt rise, with a maximum at 10 min. and a gradual decrease over a 12-hr. period (FIGURE 3). Studies are in progress to determine whether the blood glucose effect of intravenous tolbutamide varies directly and consistently with the plasma level of the drug. Unfortunately, the present method for measuring tolbutamide is not sufficiently accurate to give the precise information desired. However, insignificant amounts of tolbutamide remained in the plasma after 24 hr. Measurements of plasma levels of tolbutamide in dogs given oral tolbutamide, 25 mg./kg. or 100 mg./kg., have shown that approximately 56 and 72 hr., respectively, are necessary to free the plasma finally of the drug.[17] An overlapping effect of individual doses of intravenous tolbutamide is not a serious consideration if repeat studies are contemplated in the same individual. In order to be certain that there are no residual toxic effects of intravenous tolbutamide, and also as an extra precaution against any overlapping drug effect that might jeopardize the interpretation of the results of our studies, a minimum of 7 days was allowed to elapse before repeating any tolbutamide study.

The only untoward effects noted with acutely administered intravenous tolbutamide occurred when the material was given in concentrations greater than 10 per cent or when it was administered too rapidly. Experience has shown that in practically every instance an intravenous injection of a 10 per

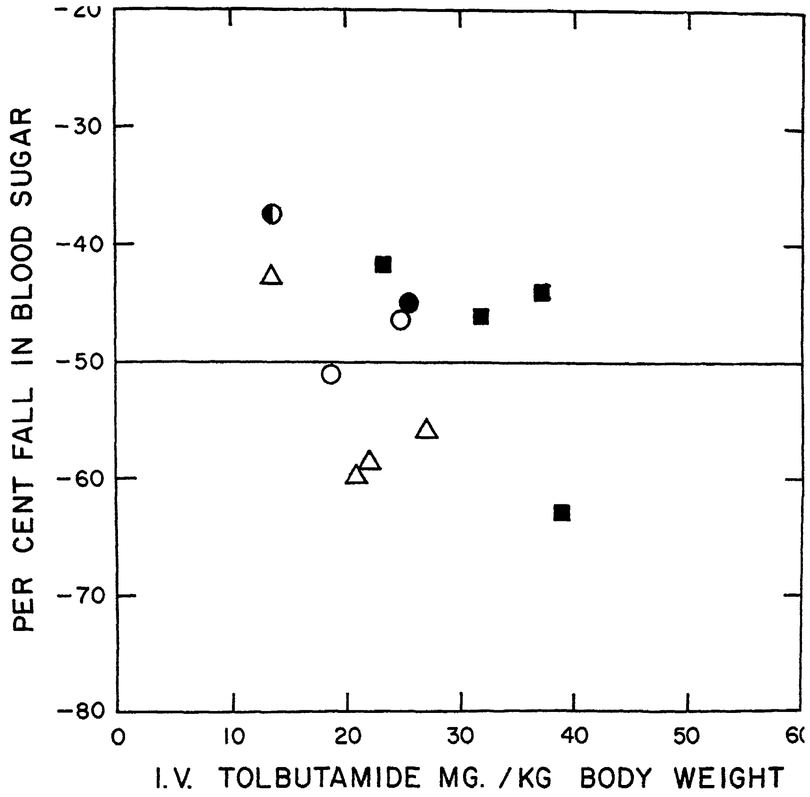

FIGURE 2. The percentage fall in blood sugar 30 min. after intravenous sodium tolbutamide. Symbols refer to individual subjects. Where the same symbol is repeated it indicates the response in this individual to different amounts of tolbutamide.

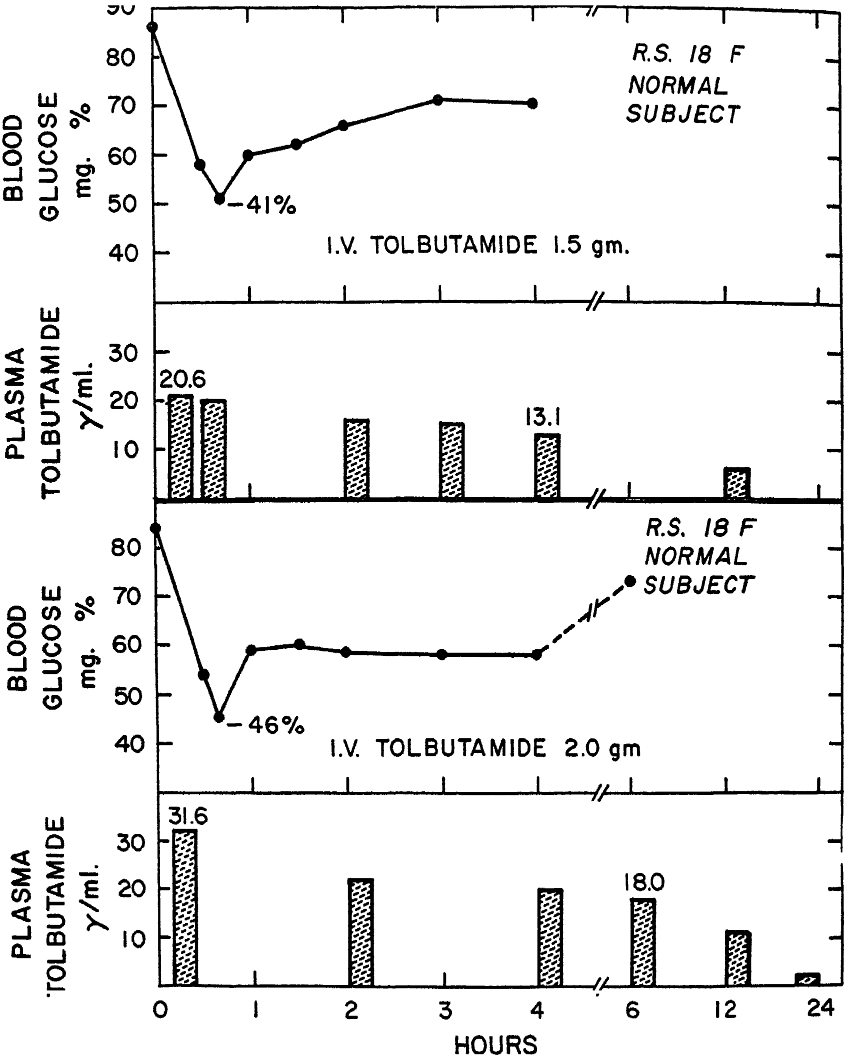

FIGURE 3. The plasma levels of tolbutamide varied with the dose administered. After 24 hr. very little material remained.

cent solution over a 3-min. period will give a comparable hypoglycemic effect without adverse symptoms. The local effect observed has been slight burning along the course of the vein in the immediate vicinity of the injection site. There has been no objective evidence of thrombophlebitis, and the venospasm has been transient. Some subjects experienced a temporary feeling of general warmth during the injection period, and one subject reported a sour taste a few seconds after starting the injection. Others noted a feeling of fullness in the head, and one subject experienced, transiently, spots before the eyes.

In no instance did any of these symptoms last longer than a few seconds after completing the injection. No evidences of leukopenia nor of renal or hepatic dysfunction have been observed in any of the subjects studied, as determined by repetitive blood counts, urinalysis, and liver function studies.

Disposition of glucose after intravenous tolbutamide. In order to evaluate the effect of tolbutamide on the disposition of glucose, comparative tests such as the insulin-glucose tolerance test and the "tolbutamide-glucose tolerance" test were employed. In the insulin-glucose tolerance test, as devised by Engel *et al.*,[8] insulin (0.1 unit per kg. body weight) is given intravenously, and 30 min. later an oral load of glucose (0.8 gm. per kg. body weight) is administered. Following this, the blood glucose rises from hypoglycemic levels; this effect represents the combination of the adaptive responses to hypoglycemia and the absorption of the oral glucose. In the test tentatively designated as the "tolbutamide-glucose tolerance test," a comparable hypoglycemic dose of sodium tolbutamide is substituted for the insulin, and the remainder of the test is performed identically.

Glucose absorption. Because carbutamide has been reported to interfere with the absorption of oral glucose in rats,[18] it seemed important to determine the effect of tolbutamide on glucose absorption in man before proceeding with tests such as the "tolbutamide-glucose tolerance test" in which oral glucose is employed. Information in this regard was inferred by comparing a glucose-insulin tolerance test with one using tolbutamide instead of insulin. In the glucose-insulin test, oral glucose (0.8 gm. per kg. body weight) and intravenous insulin (0.1 unit per kg. body weight) are administered simultaneously. In normal subjects the blood glucose tends to remain at control levels over a 90- to 120-min. interval. Presumably the amount of glucose absorbed is sufficient to maintain the blood glucose at an essentially normal level after the prescribed dose of insulin. The similarity of the results of a glucose-insulin tolerance test and a glucose-tolbutamide tolerance test is evident in FIGURE 4. The blood sugar curve in both instances remained essentially flat over the prescribed time interval, suggesting that glucose was absorbed at a rate adequate to balance the hypoglycemic effects of both insulin and tolbutamide.

However, certain differences were noted between the insulin-glucose tolerance test and the "tolbutamide-glucose tolerance" test in normal subjects. With the former test procedure, there is an "overshoot" of the blood glucose above the control level 30 to 60 min. after oral glucose is given. With the test employing tolbutamide, this "overshoot" was absent except in one subject. This suggests that one of the mechanisms participating in the elevation of the blood sugar has been affected by the tolbutamide. As noted above, our studies do not suggest that it might be due to altered glucose absorption. Measurements of plasma hydroxycorticoids showed that tolbutamide had no appreciable effect on adrenal cortical activity. Other investigators have reported no alteration in urinary 17-hydroxycorticoids or 17-ketosteroids.[19, 20] Therefore, the most plausible explanation for the failure to obtain the hyperglycemia "overshoot" may well be a decrease in glucose output by the liver. Since the subjects in our studies were normal and on a high carbohydrate

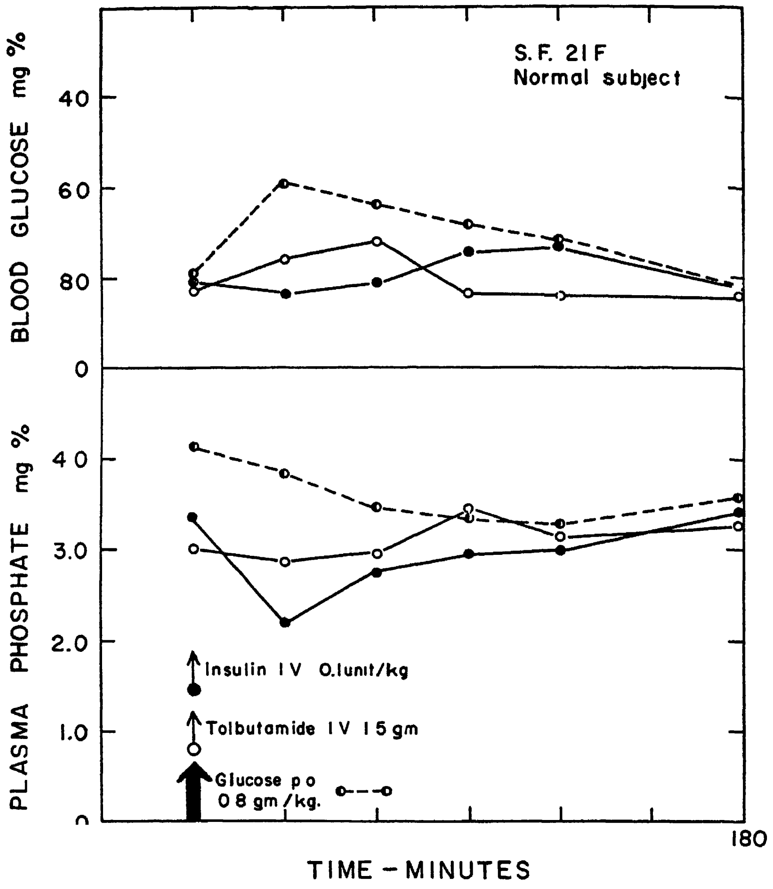

FIGURE 4. An insulin-glucose tolerance test and "tolbutamide-glucose tolerance test" are compared in the same individual. The blood sugar curve remained essentially flat after each substance. The fall in phosphorus was more marked with insulin. The control curves refer to the changes observed with administration of glucose alone (0.8 gm. per kg. body weight).

diet, there was little likelihood of depletion of liver glycogen stores. Inhibition of hepatic glycogenolysis is an alternate possibility. Support for this effect of tolbutamide has come from *in vitro* studies using tolbutamide (TABLE 1) and from other studies using, in addition to tolbutamide, the known glycogenolytic agents epinephrine[21] and glucagon.[22] Mobilization of glycogen from slices of rabbit liver seems to proceed more slowly under the influence of tolbutamide. Even in the one subject mentioned earlier who showed an increase in blood sugar over control values in a tolbutamide-glucose tolerance test, the maximum blood sugar reached was appreciably below that obtained in an insulin-glucose tolerance test.

In order to explore further the effect of intravenous tolbutamide on the

TABLE 1
PERCENTAGE DEPRESSION BY TOLBUTAMIDE OF GLUCOSE PRODUCTION BY RABBIT LIVER SLICES

Tolbutamide concentration*	Percentage depression
1.37 × 10^{-3} *M*	20
2.74 × 10^{-3} *M*	30
5.6 × 10^{-3} *M*	37.5

* The range of blood levels found to produce hypoglycemia in animals and humans is 1 × 10^{-4} *M* to 1 × 10^{-3} *M*

disposition of glucose, tests employing rapidly administered loads of glucose were performed; in these the disappearance rate of blood glucose without drug was compared with that obtained using insulin or tolbutamide. The technique employed was that described by Conard *et al.*,[9] in which 50 per cent glucose solution, 0.33 gm. per kg. body weight, is given intravenously rapidly, and blood specimens are drawn at 15-min. intervals for a 60-min.

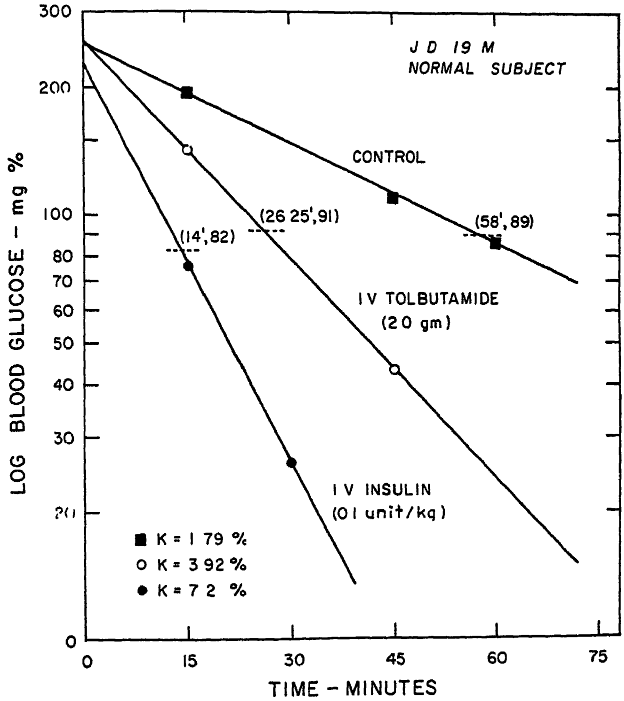

FIGURE 5. Blood glucose disappearance curves using 50 per cent glucose, 0.66 ml per kg body weight, intravenously and simultaneously with either intravenous insulin or intravenous tolbutamide. The time elapsed until the control blood sugar is reached, and the fasting blood sugar values are shown beside the exponential curves

period. The disappearance curve of the glucose is a straight line when plotted semilogarithmically. These workers as well as others[23] who have used similar glucose tolerance tests have interpreted the rate of blood glucose disappearance as being an index of insulin activity. In normal subjects Conard *et al.*[9] found a disappearance rate constant of 1.8 ± 0.16 per cent per min. After insulin and glucose together it increased to 5.87 ± 1.7, and, in one patient with hyperinsulinism it was 3.22 per cent per min. FIGURE 5 shows the results of a study employing this technique without drugs and with insulin and tolbutamide. The disappearance rate constant was 1.79 per cent per min. with glucose alone. Insulin, 0.1 unit per kg. body weight, administered simultaneously with the glucose load, increased the disappearance rate to 7.2 per cent per min. A previously established hypoglycemic dose of tolbutamide (25 mg. per kg. body weight), given intravenously with the glucose load, produced a more than twofold increase in the disappearance rate to 3.92 per cent per min. Therefore, the effect on glucose disappearance was similar to that of insulin, although less in magnitude. Other studies with sodium tolbutamide and the rapid intravenous glucose tolerance test devised by Amatuzio[23] have also revealed an increase in the rate of glucose disappearance.

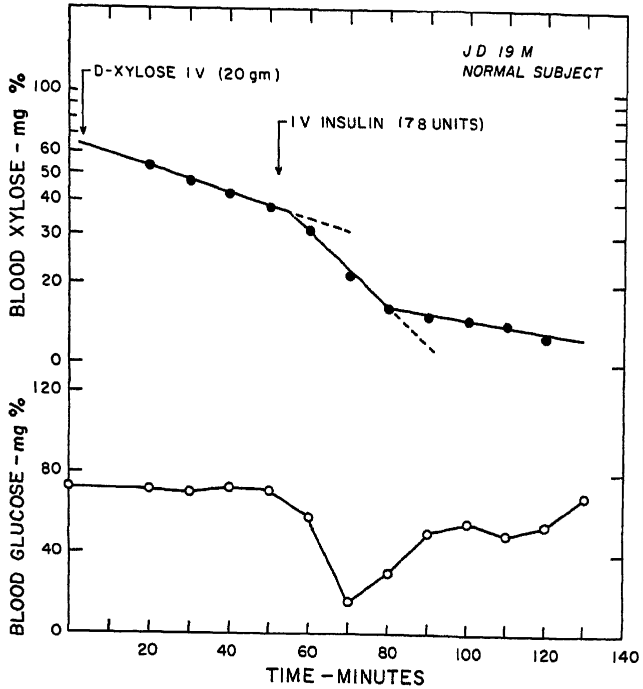

FIGURE 6. The effect of insulin on the semilogarithmic disappearance curve of infused D-xylose. Note the phase of enhanced disappearance after insulin. The blood glucose fell below 40 mg. per cent. This effect of insulin on D-xylose disappearance has been shown to be independent of insulin-induced hypoglycemia.[6]

In most instances our studies revealed a marked similarity between the effects of insulin and those of tolbutamide on glucose disposition. Changes in phosphorus also tended to be similar (FIGURE 4). In marked contrast, blood pyruvate failed to show the characteristic rise after tolbutamide which is normally seen after insulin. Preliminary studies on serum potassium also suggest a similar discrepancy. These findings, which will be the subject of a separate communication, suggested the following investigations.

Pentose studies. D-Xylose and L-arabinose were administered intravenously in 20-gm. doses over a 15- to 20-min. period. Levine *et al.*,[5] using the eviscerated dog, showed that insulin acts to increase the volume of distribution of the insulin-responsive sugars. Other studies[6] have shown that insulin acts on these sugars infused into man, and the type of response observed suggests a similar action.

Since the response of the pentoses D-xylose and L-arabinose affords another criterion of insulin activity, experiments have been performed using these compounds to contrast the effects of insulin and tolbutamide in the same individual. FIGURE 6 demonstrates the effect of insulin on the disappearance of infused D-xylose from blood. In the 30-min. period following insulin administration the xylose disappearance was tripled. A comparison of the 30-min. postinsulin xylose level with that expected from extrapolation of the initial curve reveals a 40 per cent reduction in blood level. The degree of

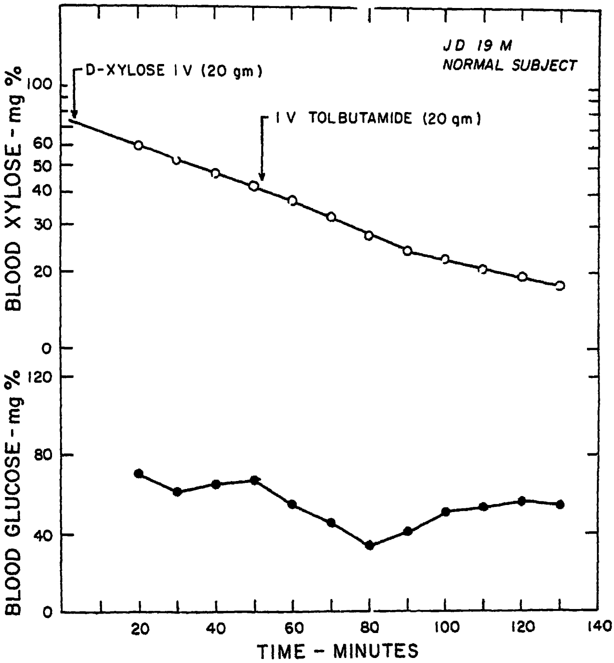

FIGURE 7. The semilogarithmic disappearance from blood of infused D-xylose after tolbutamide administration. No enhancement of disappearance is seen. With tolbutamide the blood glucose also fell below 40 mg. per cent.

enhancement of xylose disappearance compares remarkably well with the effect of insulin on glucose disappearance described above. However, the response of pentoses to insulin is independent of a change in blood glucose.[6]

A strikingly different pattern was observed with intravenous tolbutamide. This substance did not alter the disappearance curve of D-xylose (FIGURE 7) or of L-arabinose from the blood. Thus, although the blood disappearance curve of pentose was unaffected, the blood glucose fell to less than 40 mg. per cent. This is strongly suggestive of the fact that tolbutamide is devoid of an insulinlike action on pentose, although a hypoglycemic effect was exhibited, and it points to a major difference in the mechanism of action of insulin and tolbutamide.

Discussion

These experiments were designed to compare and contrast the effects of intravenous tolbutamide and intravenous insulin. From these studies certain inferences as to the mechanism of sulfonylurea-induced hypoglycemia were drawn. The alteration in blood glucose that follows the administration of either of these substances is strikingly similar with respect to time of onset and degree of hypoglycemia, provided care is taken to determine a precisely comparable dose of tolbutamide. However, in contrast to insulin, tolbutamide causes a delay in return of the blood glucose to control levels. Mirsky and his co-workers[16] have observed this same effect in rats and have concluded that the sustained hypoglycemia is due to prolongation of the action of endogenous insulin brought about by the sulfonylurea. Although our findings in this regard cannot disprove this opinion, the results with glucose and pentose suggest alterations in normal adaptive processes as a basis for the slow return of blood glucose to normal after tolbutamide. It is thought that this alteration of homeostasis has as a specific basis a disturbance in the liberation of glucose from adequate glycogen stores. As mentioned before, our *in vitro* studies with rabbit liver slices and tolbutamide, as well as investigations of others, are consistent with such an interpretation. Until more precise measurements of blood levels of insulin after tolbutamide are made, it will not be possible to do more than speculate as to the correct interpretation.

Although altered glucose absorption could never be the prime factor in explaining the hypoglycemic effect of tolbutamide, it is of considerable clinical importance. Since there is no evidence in these studies to suggest any delay or impairment in this regard, the development of a severe or protracted hypoglycemia in subjects receiving treatment with tolbutamide could presumably be treated effectively by the administration of oral glucose.

The effect of tolbutamide on phosphorus was similar to that of insulin on this element. A prompt lowering of phosphorus and a gradual return to control or above control levels occurred with both tolbutamide and insulin. In a few instances with tolbutamide, following the initial hypophosphatemia, phosphorus appeared to rise higher than with insulin but, contrary to the observations of Mohnike and Bibergeil,[25] this finding was not uniform. After insulin also, high phosphorus levels may be noted if measurements are continued for sufficient time. The lack of the anticipated rise in blood pyruvic acid after tolbutamide is in direct contrast to that seen after insulin. As mentioned before, preliminary studies indicate that the changes in serum potassium also differ with the two compounds.

The studies of blood pentose disappearance are critical to the proposed theorem. The disappearance rate of the insulin-responsive sugars was unaltered by tolbutamide, and D-xylose and L-arabinose could well be termed "tolbutamide-unresponsive pentoses." Because the alteration in disappearance rates of these compounds following the administration of insulin is thought to represent increased peripheral insulin activity,[6] these studies suggest that the sulfonylureas do not act primarily by enhancing the peripheral action of endogenous insulin. It might reasonably be suggested that our failure to observe an effect with tolbutamide was due to insufficient circulating insulin. In other words, the release of insulin due to the tolbutamide or the enhancement of circulating insulin was not equal to the amount of exogenous insulin shown to alter the disappearance rate of certain pentoses. It should be noted, however, that the degrees of hypoglycemia and of hypophosphatemia following either insulin or tolbutamide were comparable, thereby at least suggesting that these effects of each compound were equivalent. Furthermore, other studies have shown that both tolbutamide and insulin produced a greater than twofold increase in disappearance of blood glucose. Therefore, the possibility that inadequate circulating endogenous insulin exists following tolbutamide in these pentose studies does not seem likely. The increase in peripheral glucose A-V difference known to follow insulin administration has not been demonstrated with tolbutamide.[26] This is further evidence of a lack of a peripheral effect of tolbutamide.

The disparity between the experimental results noted above suggested the necessity for a revision of existing concepts of the action of tolbutamide and the possibility that it might be difficult to reconcile these results with a single mode of action of this compound. In the normal individual the liver does not appear to be a major site of insulin activity. In contrast to this, the data presented here and from other laboratories[19, 21, 26 27] point to a distinct and significant effect of the sulfonylureas on hepatic glucose metabolism. More specifically, the sulfonylureas appear to produce a defect in glucose release and synthesis (as judged by increased hepatic glycogen), defective hepatic glycogenolysis, and the impaired conversion of both fructose and galactose to glucose. Could these effects have a common background? If the machinery in the liver concerned with glucose metabolism were in some unexplained manner made more responsive to endogenous insulin, would the observed findings be consistent with such an hypothesis? In this schema the potentiating effects of tolbutamide on endogenous insulin would be in terms of an enhanced or synergized hepatic effect rather than in terms of alterations in peripheral insulin activity. Support for this view is derived from the studies of Anderson and his co-workers,[27] who found that sodium tolbutamide (150 mg./kg. I.V.) administered to an anesthetized dog caused, within 10 min., a 28 per cent drop in the output of glucose into the hepatic vein, and those of Purnell *et al.*[26] who observed, likewise in dogs, a fall in hepatic as well as in portal glucose concentration after the administration of tolbutamide. Furthermore, the lack of effect of tolbutamide on peripheral glucose A-V differences[26, 27] and the pentose studies already described do not suggest that tolbutamide increases peripheral insulin activity. If this were true one might reason teleologically that tolbutamide causes an increase in hepatic glycogen and/or a decrease in glucose release and/or synthesis. The lack of rise in blood pyruvic acid after tolbutamide, as well as the initial fall observed by us[24] and by others,[28] might be due to the channeling of 3-carbon fragments such as pyruvic acid into the liver for glycogenesis. Studies are in progress using pyruvic acid to determine this possibility. Similarly, the changes noted in the blood glucose disappearance rate could also be interpreted as resulting from increased hepatic uptake of glucose. Parenthetically, attention is directed to this latter observation since it suggests that, contrary to accepted beliefs, the rate of blood glucose disappearance is the resultant of a number of simultaneous processes and not only of peripheral insulin activity. More specifically, it points toward enhanced hepatic glucose metabolism as an important mediator of the observed effect.

Some workers have suggested that the sulfonylureas might act by altering endogenous insulin chemically, in some unknown way.[29] If this should prove correct, it would be consonant with the above hypothesis, in that such a chemical alteration might be the cause of the increased hepatic effect of endogenous insulin.

This proposed concept of an increased hepatic effect of endogenous insulin resulting from sulfonylurea administration might be investigated profitably by using hepatectomized animals and by measuring the effect of endogenous insulin on hepatic glucose metabolism directly. One study to be reported in this symposium is not in agreement with this concept of an enhanced hepatic insulin effect.[30] However, as with insulin, the variabilities in response of many animal species point up the fact that caution must be exercised in interpreting animal data in terms of man.

Summary

Intravenously administered tolbutamide has been shown to produce hypoglycemic effects comparable to those of insulin. The effects on blood glucose, blood glucose disappearance rate, and on blood phosphorus suggest an insulin-like action. Studies of the effect of this compound on the disappearance rate of insulin-responsive pentoses suggest that there is no enhanced peripheral activity of insulin as judged by this criterion; other previously reported studies support this view. We propose a concept of the mechanism of the hypoglycemic action of tolbutamide according to which some of its hypoglycemic effect is attributed to an enhancement of the action of endogenous insulin on the liver without a similar proportional effect on peripheral insulin activity.

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EFFECTS OF TOLBUTAMIDE AND INSULIN ON FRUCTOSE AND GLUCOSE METABOLISM IN DIABETES MELLITUS*

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Despite intensive study the locus of action of tolbutamide on carbohydrate metabolism in man is unknown. Evidence obtained by direct infusions into the pancreas in animals strongly suggests that the drug stimulates the secretion or release of insulin by the β cells. On the other hand, there is equally strong evidence to indicate that some metabolic effects of tolbutamide are different from those observed following subcutaneous or intravenous injection of insulin. The present report describes differences in fructose and glucose metabolism in diabetic patients given tolbutamide and insulin.

Subjects and Methods

Diabetic patients were divided into four groups: (1) "obese" diabetes ("adult," nonketotic, noncatabolic); (2) "thin" diabetes ("juvenile," ketotic, catabolic); (3) "steroid" diabetes (due to hyperfunction of the pituitary-adrenal system); and (4) "pancreatic" diabetes (due to destruction of the pancreas by surgery or disease).

The techniques and methods used have been described previously.[1, 2] Each patient was admitted to the metabolic ward. Insulin injections were stopped, and studies were delayed until his metabolism had reverted to its abnormal untreated state. Tolbutamide response tests were begun after a fourteen-hour overnight fast, unless otherwise indicated. Three to five grams of tolbutamide were given by mouth, followed by venous blood sampling at hourly intervals for eight hours. In each case on a different day exactly similar observations were made without giving tolbutamide.

In the second group of experiments a steady absorptive state was maintained by continuous tube feeding.[2] A nutritionally balanced synthetic liquid diet containing glucose or fructose as the sole carbohydrate was delivered through a tube into the gastrointestinal tract at a constant rate day and night.

Results

In both the "obese" and "steroid" groups of diabetic patients tolbutamide uniformly produced a decrease in the level of glucose in the blood. In the "thin" and in the "pancreatic" groups of diabetic patients it had no effect. Tolbutamide therefore caused a response only in those diabetic patients whose plasma contains measurable amounts of insulin.[3] Typical

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tolbutamide response tests in an obese diabetic patient without ketosis, and in a thin diabetic patient with ketosis, are shown in FIGURES 1 and 2 respectively.

A previous report[2] describes studies on the metabolism of glucose and fructose in patients with untreated diabetes while they were maintained in a steady absorptive state by continuous tube feeding. Either glucose or fructose constituted the sole dietary carbohydrate. In a group of "thin" patients with "ketotic" diabetes, constant feeding of glucose caused elevation of the level of glucose in the blood and did not influence ketosis and nitrogen loss. Constant feeding of fructose alleviated the ketosis and other metabolic abnormalities, while permitting the blood glucose values to return to the fasting level. No fructose was found in the peripheral blood. FIGURE 3 illustrates the blood glucose changes in three patients from this group. These patients did not respond to tolbutamide.

Patients with nonketotic "obese" diabetes reacted differently. Changes in blood glucose during continuous tube feeding in such a patient are shown in FIGURE 4. The blood glucose level increased to a marked degree during constant feeding of glucose, but it failed to return to the fasting level during constant feeding of fructose. The tolbutamide response was completely absent during constant glucose feeding, but was restored during constant

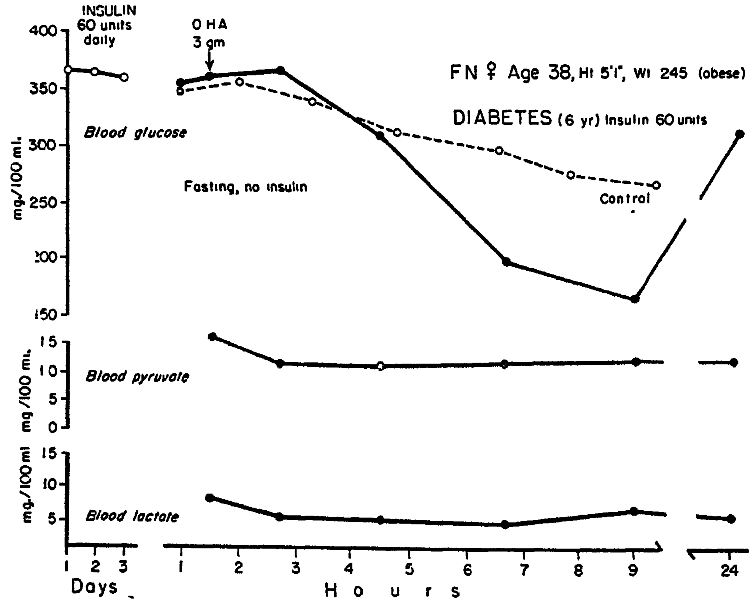

FIGURE 1. The effect during fasting of 3 gm. of tolbutamide by mouth on the blood glucose level in an obese patient with nonketotic diabetes. Stopping the daily injection of 60 units of NPH insulin did not alter the blood glucose level, but after tolbutamide it decreased from 360 to 160 mg. per 100 ml. within 8 hours. The interrupted line illustrates the change during a similar fast on a different day without medication. Changes in blood pyruvate and blood lactate are discussed elsewhere.[1] (Reproduced by courtesy of the editor of *Metabolism*.)

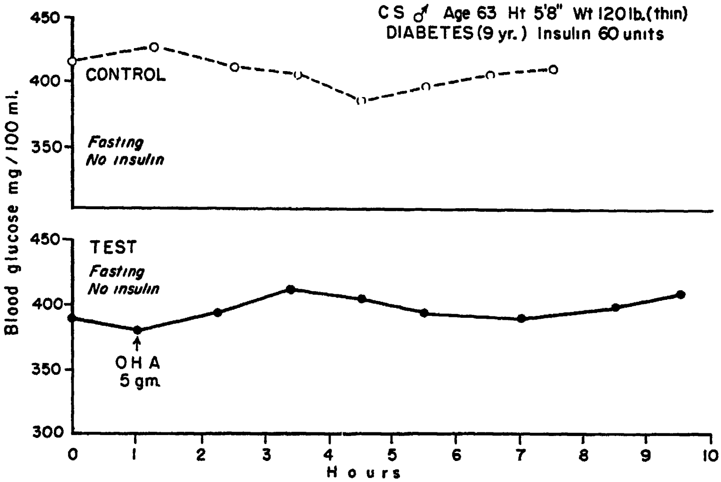

FIGURE 2. The effect during fasting of 5 gm. of tolbutamide by mouth on the blood glucose level of a thin patient with ketotic diabetes. The values for blood glucose remained constant both during a control fasting period without medication (interrupted line) and after tolbutamide (solid line). (Reproduced by courtesy of the editor of *Metabolism*.)

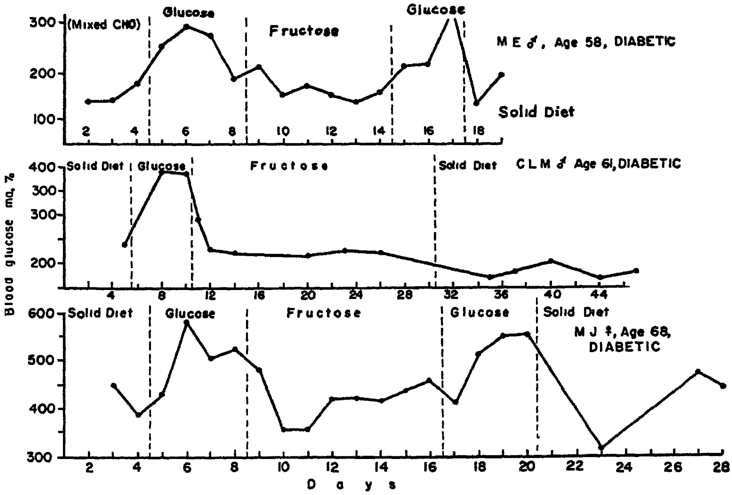

FIGURE 3. The blood glucose levels found during fasting, during continuous glucose tube feeding, and during continuous fructose tube feeding in three patients with ketotic diabetes. During the periods of tube feeding, a nutritionally balanced synthetic liquid diet containing either glucose or fructose as the sole carbohydrate was delivered into the stomach at a constant rate "around the clock." In the initial period, morning fasting blood glucose values were obtained while the patient consumed a three-meal-per-day solid diet that was isocaloric with the tube feeding to follow. When continuous tube feeding with glucose as the only carbohydrate was given, the level of glucose in the blood increased. However, when fructose was substituted for glucose in the tube feeding, the level of glucose in the blood in each case returned to the value found during fasting.

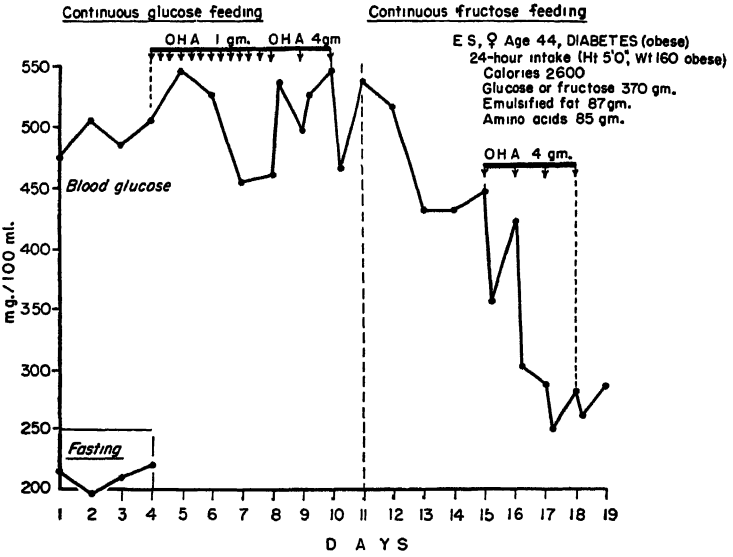

FIGURE 4. The effects during continuous tube feeding of 4 gm. of tolbutamide by mouth on the blood glucose level in an obese patient with nonketotic diabetes. A nutritionally balanced synthetic liquid diet containing either glucose or fructose as the sole carbohydrate was delivered into the stomach at a constant rate "around the clock." During a preliminary period of study on a solid diet the morning fasting blood glucose value was approximately 200 mg. per 100 ml. When continuous tube feeding containing glucose as the only carbohydrate was given, the level of glucose in the blood increased to approximately 500 mg. per 100 ml. Daily doses of tolbutamide had no effect on blood glucose values during this period. When fructose was substituted for glucose in the tube feeding, the level of glucose in the blood decreased only slightly, and remained far above the fasting value. However, daily doses of tolbutamide caused the blood glucose level to decrease sharply from 450 to 250 mg. per 100 ml. (Reproduced by courtesy of the editor of *Metabolism*.)

fructose feeding. FIGURE 5 illustrates the response of another patient with "nonketotic" diabetes to single doses of tolbutamide and insulin during fasting, during constant glucose feeding, and during constant fructose feeding. It can be seen that, after the oral administration of tolbutamide, the blood glucose level decreased during fasting and during constant fructose feeding, but did not change during constant glucose feeding. After the injection of insulin, the blood glucose level decreased equally during glucose and fructose feeding.

Discussion and Conclusions

Only those patients who retain the ability to produce insulin[3] respond to tolbutamide. This suggests that tolbutamide lowers the blood glucose level by stimulating the release of insulin or by potentiating the action of insulin. Experiments employing perfusion of the pancreas[4] and cross-circulation techniques[5] appear to establish the existence of the former mechanism beyond reasonable doubt.

However, if the action of tolbutamide is mediated by insulin release or

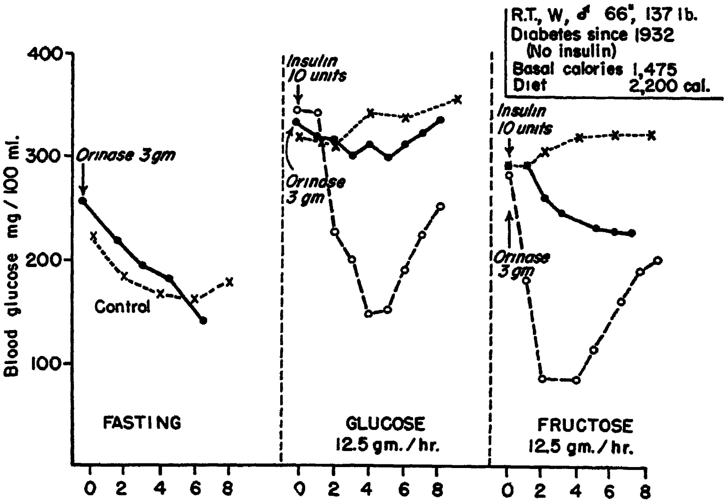

FIGURE 5. The effects during fasting, during continuous glucose tube feeding, and during continuous fructose tube feeding, of tolbutamide (Orinase) by mouth and of insulin by injection on the blood glucose level in a patient with nonketotic diabetes. During the periods of tube feeding, a nutritionally balanced synthetic liquid diet containing either glucose or fructose as the sole carbohydrate was delivered into the stomach at a constant rate "around the clock." The dotted line (x ------- x) represents the blood glucose values found during control periods, without medication. The solid lines (●———●) represent values found after giving 3 gm of tolbutamide by mouth, and show that the blood glucose level decreased during fasting and during continuous fructose tube feeding, but not during continuous glucose tube feeding. The interrupted lines (O— — — O) represent values found after the subcutaneous injection of 10 units of soluble insulin, and show that the blood glucose level decreased both during continuous glucose tube feeding and during continuous fructose tube feeding.

potentiation, its metabolic effects should be exactly the same as those of insulin. They are not. Tolbutamide does not improve glucose tolerance,[6] does not accelerate glucose oxidation,[7] and does not increase pyruvate formation.[8] The present report shows that, unlike insulin, its effectiveness disappears during glucose absorption. This finding suggests that tolbutamide principally influences the output of glucose by the liver.[9] During constant glucose absorption, hepatic glucose release is already inhibited physiologically;[10] during constant fructose absorption, the liver must synthesize and release glucose to supply the needs of the body.

There is, therefore, convincing evidence to show that tolbutamide stimulates the release of insulin from the pancreas. There is equally convincing evidence to indicate that many of its metabolic effects are different from those produced by the injection of insulin. These effects can be explained by an action on the liver. These two modes of action are not mutually exclusive. The emphasis different investigators place on the primacy of one action or the other may be due to differences in routes of administration of the drug, to the use of different doses, or to different metabolic responses in the species of animals studied. However, it is possible that endogenous insulin released into the portal circulation may not have the same effects as exogenous insulin entering the peripheral circulation. If this is true, the possibility exists that all the effects of the drug may be the result of release of endogenous insulin into the liver via the portal vein.

Acknowledgment

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THE ROLE OF INSULINASE IN THE HYPOGLYCEMIC RESPONSE TO SULFONYLUREAS*

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The hypoglycemic response of the healthy animal or man to the administration of a single dose of tolbutamide (Orinase) is divisible into two phases: (1) an initial phase lasting less than one hour, during which a maximum decrease in the blood sugar concentration occurs; and (2) a subsequent phase of restitution, lasting a variable period of time, during which the blood sugar is gradually restored to its initial concentration (FIGURE 1). With an oral dosage of less than 50 mg. tolbutamide per kilogram of body weight, both phases of the response are dependent upon the dosage. Larger dosages, however, do not increase the initial phase, but may decrease the rate at which the blood sugar concentration is restored to its initial level.[1]

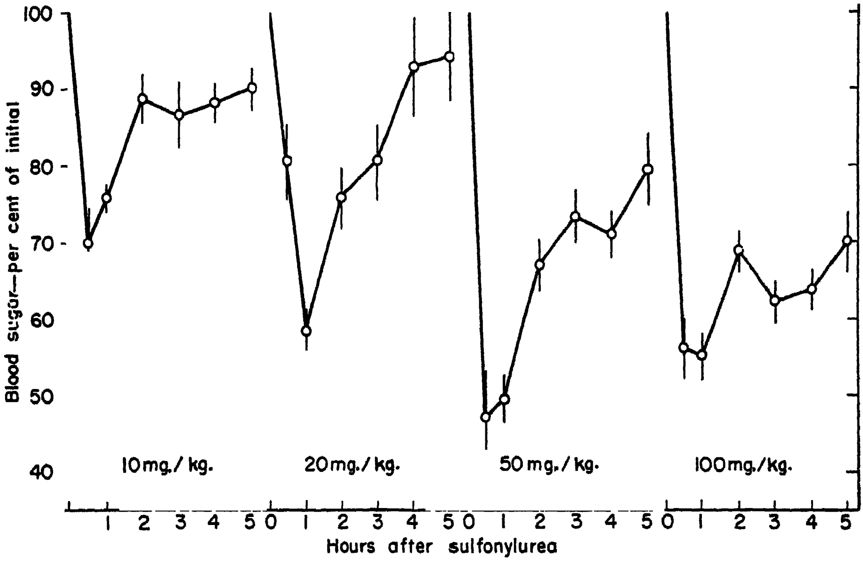

FIGURE 1. The hypoglycemic response of man to various doses of tolbutamide by mouth. The blood sugar concentration at each interval is expressed as the percentage (mean ± S.E.) of the concentration immediately before the tolbutamide by mouth. Reproduced from Diengott and Mirsky[1] (by courtesy of the *Journal of Pharmacology and Experimental Therapeutics*).

Destruction or removal of the β cells of the pancreas prevents the hypoglycemic response to the aryl sulfonylureas in man,[2, 3, 4] dog,[5, 6, 7, 8] rat,[9, 10] rabbit,[8, 11] and toad.[7] Ducks and chickens, however, develop the same hypoglycemic response in the presence or absence of the pancreas.[8] Thus, in

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all species except the fowl, the hypoglycemic response to tolbutamide cannot be due to an insulin-independent glycogenic or glycogenolytic mechanism, since such could occur also in the absence of the pancreas. All the available evidence indicates that the initial phase of the hypoglycemic response of most species is dependent upon the direct stimulation of the β cells and the discharge of insulin into the circulation from pancreatic stores.[12, 13]

In accord with this hypothesis is the demonstration that factors that reduce the concentration of extractable insulin in the pancreas also result in a decrease in the degree and rapidity with which the hypoglycemia develops. Thus, the daily administration of from 2 to 3.5 mg. growth hormone per kilogram of body weight to dogs for 6 to 8 days results in an extensive degranulation of the β cells of the islets of Langerhans and a reduction in the concentration of extractable insulin in the pancreas; this may occur even before the development of hyperglycemia.[14] The same treatment prevents the initial rapid hypoglycemic phase that otherwise occurs in the dog given tolbutamide by mouth (FIGURE 2). Similarly, fasting, which results in a decrease in the

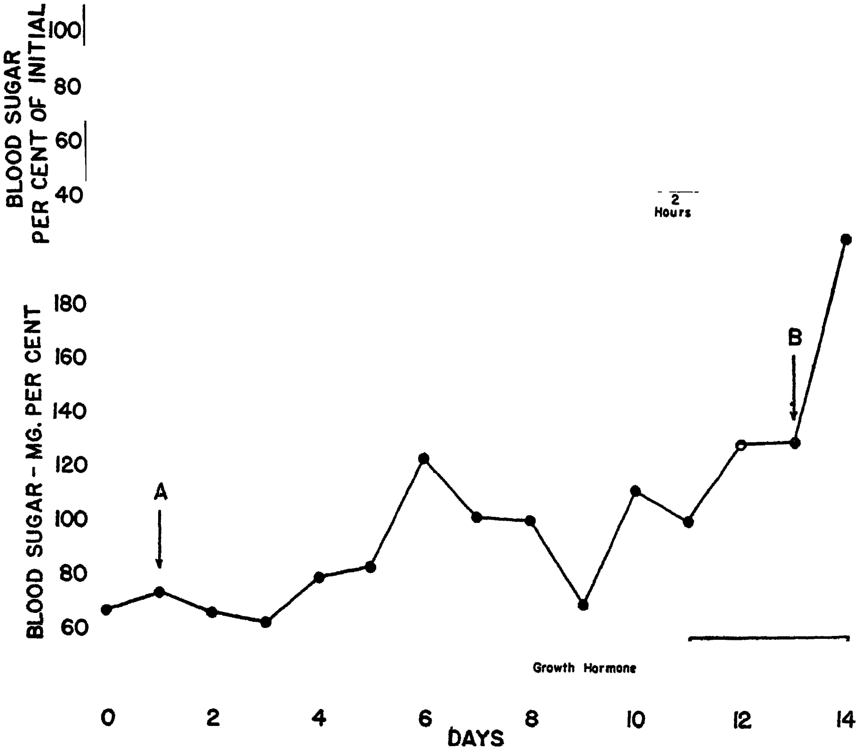

FIGURE 2. Effect of growth hormone on the hypoglycemic response of the dog to tolbutamide. Inserts depict the response to the intravenous injection of 25 mg. tolbutamide per kilogram body weight before (A) and during (B) the intramuscular administration of from 5 mg. (fourth to tenth day) to 7 mg. (eleventh to fourteenth day) growth hormone per kilogram body weight.

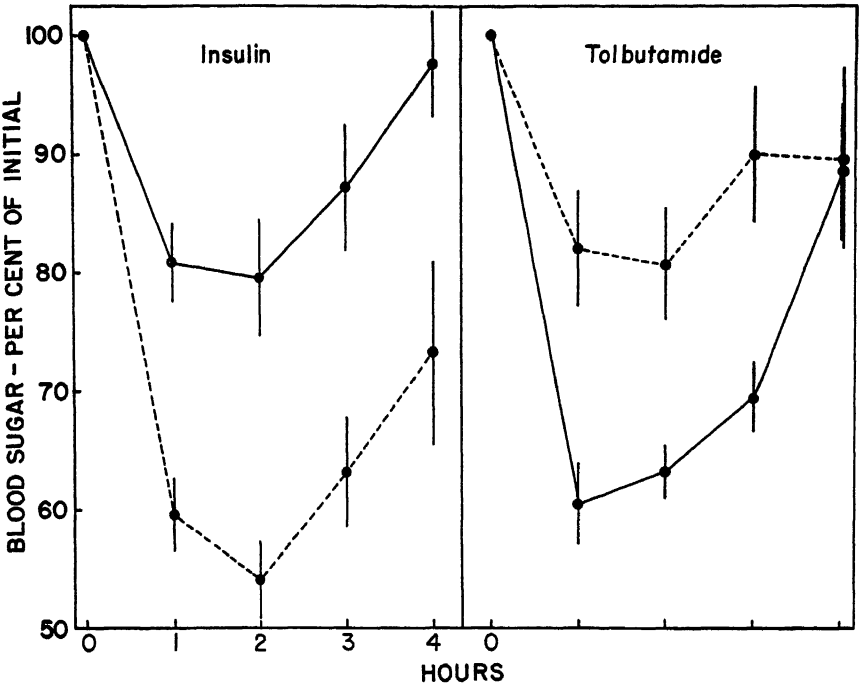

FIGURE 3. Effect of fasting on the hypoglycemic response to insulin and to tolbutamide. The blood sugar concentration (mean ± S.E.) at each interval is expressed as the per cent of the pretreatment level. Insulin (0.5 units per kilogram) administered intraperitoneally at 0 time to 20 rats after overnight fast (●——●) and to 25 rats after a fast of 8 days (●— — —●). Tolbutamide (50 mg.) administered by stomach tube at 0 time to 20 rats after overnight fast (●——●) and to 15 rats (●— — —●) after a fast of 8 days.

amount of insulin that is extractable from the pancreas,[15] results also in a decrease in the hypoglycemic response to tolbutamide by mouth (FIGURE 3). The decreased response of the fasted rat is not due to a resistance to the action of insulin, since similarly fasted rats show an increased response to a standard dose of exogenous insulin.[16] Further, whereas the alloxanized rat or rabbit with severe diabetes does not respond to tolbutamide, the alloxanized rat or rabbit with mild or moderately severe diabetes responds with a gradual decrease in the blood sugar concentration rather than with the rapid initial fall observed in the normal animal (FIGURE 4). Finally, the majority of patients who develop diabetes mellitus after the age of 40 years ("maturity onset" type) have significant, though diminished, concentrations of extractable insulin in the pancreas.[17] Such patients respond to the tolbutamide by mouth only with a slow progressive decrease in the blood sugar concentration rather than with the rapidly developing initial phase observed in healthy subjects.[18]

A rapid discharge of insulin from the pancreas into the circulation, however, cannot account for the restitution phase of the hypoglycemic response to the sulfonylureas. Thus, in healthy subjects the intravenous injection of 0.1 unit insulin per kilogram of body weight produces the same immediate response in the blood sugar concentration that occurs after the ingestion of 50 mg. tolbutamide per kilogram body weight (FIGURE 5). In both instances the same degree of hypoglycemia is attained in approximately 30 minutes. Thereafter, however, the responses differ markedly in that the blood sugar returns to the range of the initial concentration within 2 hours after the insulin, while it remains significantly below the initial concentration at 5 hours after the tolbutamide.

The difference between the response to exogenous insulin and the response to tolbutamide can be attributed to a decrease in the rate of destruction of the insulin that is discharged into the circulation. Such a decrease would ensue if tolbutamide inhibited the action of insulinase. It has been demonstrated that insulinase can be inhibited *in vitro* and *in vivo* by the aryl sulfonylureas. Thus, there is a marked reduction in the insulinase activity of the livers of rats in one hour after the administration by mouth of dosages of tolbutamide that induce hypoglycemic responses (FIGURE 6).[19] Similar

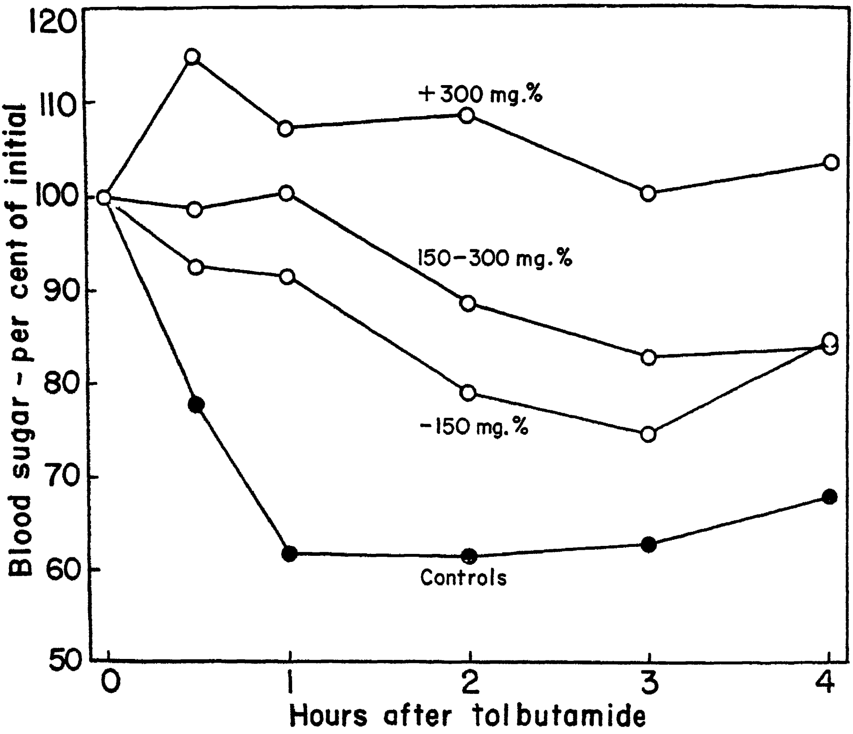

FIGURE 4. Effect of tolbutamide on the blood sugar of alloxanized rabbits. The alloxanized animals (○———○) were grouped as follows: 8 animals with fasting blood sugars of less than 150 mg. per cent, 8 with blood sugars between 150 and 300 mg. per cent, and 6 with values above 300 mg. per cent. Nine untreated rabbits served as controls (●- - ●).

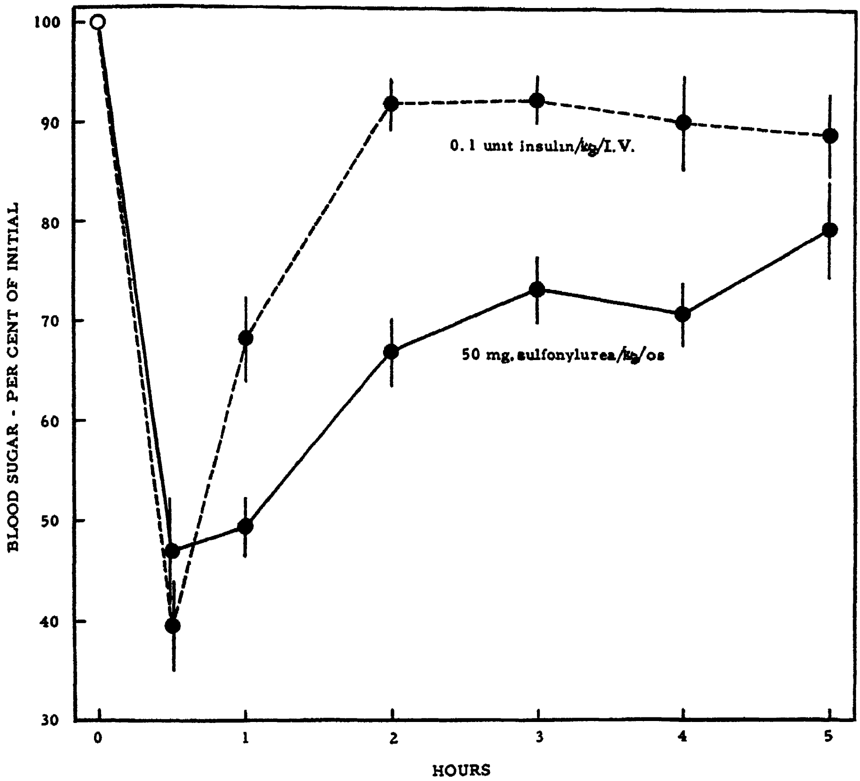

FIGURE 5. Comparison of the hypoglycemic responses of man to the intravenous injection of insulin and to tolbutamide by mouth. The blood sugar concentration at each interval is expressed as the per cent (mean ± S.E.) of the concentration immediately before the administration of insulin or tolbutamide. Reproduced from Diengott and Mirsky[1] (by courtesy of the *Journal of Pharmacology and Experimental Therapeutics*).

data have been obtained more recently in the chicken (FIGURE 7). It is pertinent to note that the reduction in the insulinase activity of the liver occurs at about the beginning of the restitution phase of the hypoglycemic response.

In accord with the *in vivo* studies, we found that the insulinase activity of a fresh standard extract of rat liver was inhibited *in vitro* in the presence of 3.5×10^{-2} *M* tolbutamide.[14] This was apparently confirmed by Williams.[21] Subsequently, Vaughan[22] reported that from 5×10^{-4} to 3×10^{-3} *M* tolbutamide produced no effect on the insulinase activity of whole homogenates of rat livers. More recently, Williams and Tucker[23] reported that the concentration of tolbutamide required to produce an inhibition of the insulin-degrading system of the liver was in excess of 10^{-3} *M*, yet an inhibition of almost 50 per cent was obtained with approximately 1.6×10^{-3} *M**. In view of the above, we repeated our studies and found that the heat-labile

* Computed from FIGURE 1 in Williams and Tucker.[23]

system that is responsible for the destruction of insulin by fresh extracts of rat and duck livers is inhibited by tolbutamide in concentrations as low as 5×10^{-4} *M* (FIGURE 8). The heat-stable factor in liver extracts that apparently deiodinates I^{131}-labeled insulin[20] is not affected appreciably by tolbutamide. The concentrations used in this study are within the range of those found in the circulation one hour after the administration of tolbutamide in doses that produce hypoglycemia. The apparent discrepancy between the data reported herein and those of others may be due to differences in the potency of insulinase extracts. Thus, an extract that is rich in the heat-stable factor and poor in the heat-labile factor will show very little inhibition in the presence of sulfonylurea.

If an inhibition of insulinase is involved in the hypoglycemic action of the sulfonylureas, then the administration of tolbutamide should induce a decrease in the insulin requirements of the completely depancreatized dog and an enhancement of the hypoglycemic action of exogenous insulin. Such

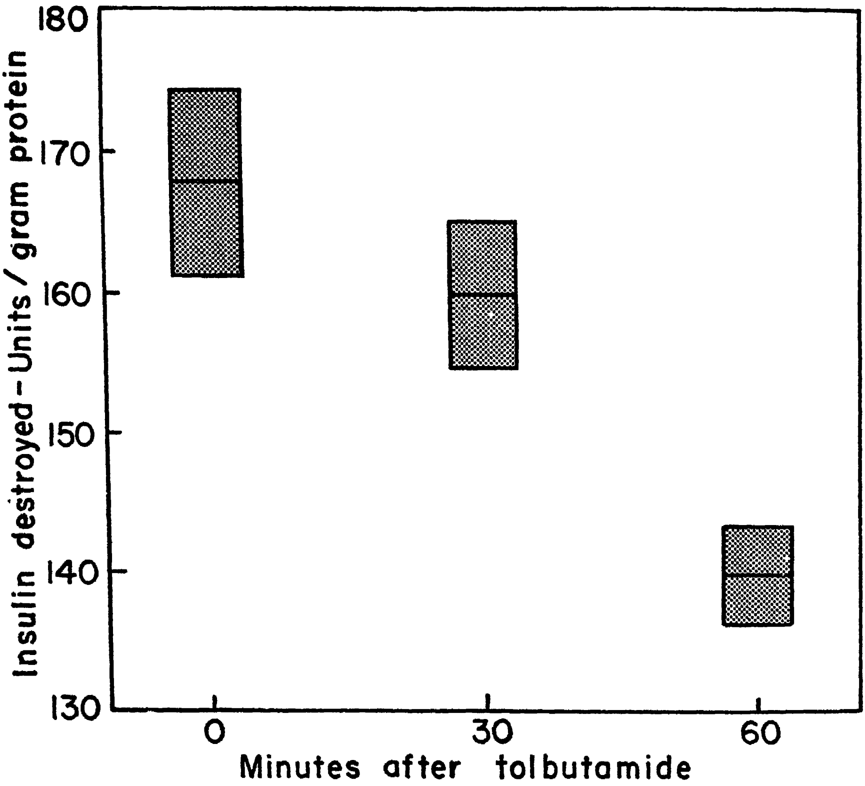

FIGURE 6. The insulinase activity of extracts of livers from rats given tolbutamide by gavage. Rats given gavage of 100 mg. tolbutamide per kilogram body weight. Livers removed from 10 rats at each designated period, standard extracts prepared from each liver and assayed.[20] The insulinase activity expressed as units of insulin destroyed per 100 gm. protein (mean ± S.E.).

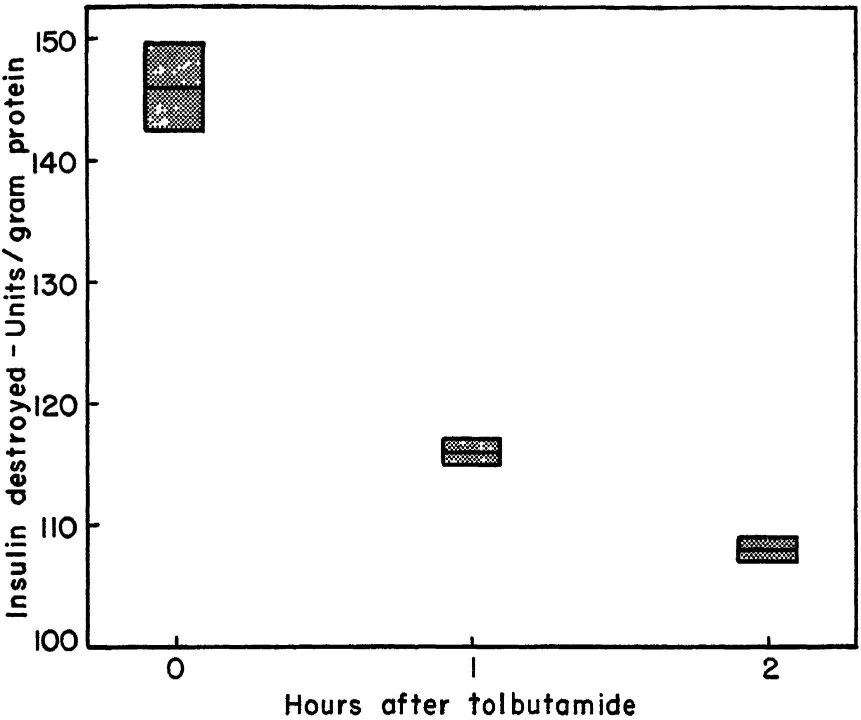

FIGURE 7. The insulinase activity of extracts of livers from chickens given tolbutamide intravenously (see FIGURE 6).

a decrease in insulin requirements has been reported by Campbell[24] and by Sirek and Sirek.[25] Contrary results have not been reported. Although it has been claimed that the sulfonylureas do not enhance the hypoglycemic action of exogenous insulin in the depancreatized dog,[6] the data reported by Houssay provide a decisive demonstration that such an enhancement can be produced.[26] Likewise, an enhancement of the hypoglycemic action of exogenous insulin can be demonstrated in patients with diabetes mellitus who do not respond to the sulfonylureas alone. Whereas the hypoglycemic response to the intravenous injection of a standard dose of insulin is essentially the same when a second dose is given four hours after the first, a marked enhancement of the response ensues if the second dose of insulin is preceded by the ingestion of tolbutamide.[27]

The studies reviewed herein make it appear probable that the initial hypoglycemic response to the tolbutamide is due to stimulation of the β cells of the islets of Langerhans and the discharge of insulin into the circulation, while the prolongation of the hypoglycemia is due, in part at least, to an inhibition of insulinase and a consequent decrease in the destruction of the endogenous insulin. This hypothesis does not preclude the possibility that additional mechanisms may be involved in the production of both phases of

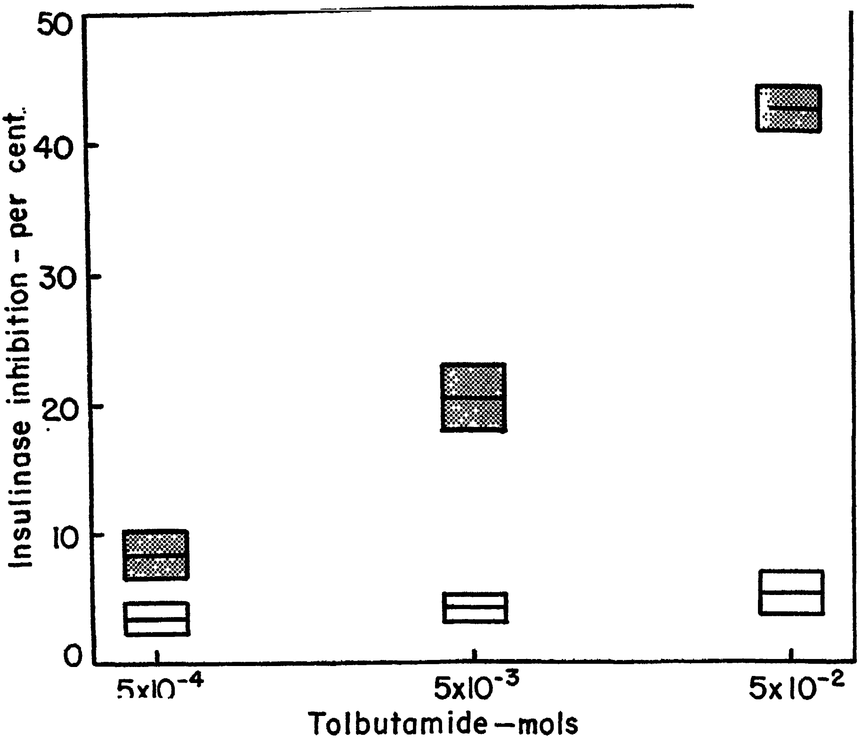

FIGURE 8. Effect of tolbutamide *in vitro* on the insulinase activity of extracts of rat livers. Ten standard extracts prepared with 0.02 *N* sodium bicarbonate.[20] 1 ml. unboiled and 1 ml. boiled extract each incubated with 1 ml. MacIlvaine's buffer containing I^{131}-labeled insulin and no or different concentrations of tolbutamide for 30 minutes at *p*H 7.8 and 37°C. Rate of release of trichloroacetic acid-soluble radioactivity determined and the per cent of inhibition (mean ± S.E.) produced by tolbutamide computed for the heat-labile insulinase (stippled bars) and the heat-stable factor (blank bars).[20]

the hypoglycemic response to the sulfonylureas. Such additional mechanisms must be effective in the fowl where the hypoglycemic response to the sulfonylureas is independent of the presence of the pancreas.

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THE EFFECT OF TOLBUTAMIDE ON GLUCOSE PRODUCTION BY THE LIVER *IN VITRO*

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The concentration of glucose in the blood in the fasting state may be altered by changes in the rate of hepatic production and/or of peripheral utilization of glucose. Most of the available evidence indicates that peripheral utilization of glucose is not enhanced by doses of the sulfonylureas that cause hypoglycemia. The glucose tolerance curve is not affected,[1-5] nor is the oxidation of C^{14}-labeled glucose accelerated in the eviscerated rabbit.[6] It has been demonstrated[7, 8] that the injection of these drugs directly into the pancreatic circulation causes the release of an insulinlike substance from the pancreas. However, the effects of the sulfonylureas in the whole animal differ in many ways from those of insulin, and there is little evidence that they possess an insulinlike action or are able to increase the amount, or potentiate the effect, of insulin in the peripheral circulation. The decrease or absence of the hypoglycemic action in alloxan diabetic or totally pancreatectomized animals has been interpreted as an indication that the sulfonylureas exert their effects through stimulation of insulin release. However, it is equally possible that the secondary derangements of metabolism that occur in the tissues of insulin-deficient animals either prevent or mask the effects of these drugs.

On the other hand, effects of the sulfonylurea derivatives on the hepatic release of glucose have been demonstrated in a variety of ways. In humans and in dogs, during fasting and after fructose or galactose administration, tolbutamide causes a decrease in the output of glucose from the liver.[1, 5, 9, 10] The rate of glucose release from liver slices incubated *in vitro* is diminished when tissue is obtained from rabbits pretreated with tolbutamide.[11] In addition, it has been observed that liver glycogen levels are higher in rats given tolbutamide than in untreated animals.[11-13] In a series of *in vitro* experiments we have explored the effects of tolbutamide on some of the reactions involved in the conversion of liver glycogen to blood sugar.

Liver glucose-6-phosphatase activity. Our attention was first directed toward a possible effect of tolbutamide on the activity of liver glucose-6-phosphatase. As reported previously,[14] we were able to demonstrate no effect of tolbutamide at a concentration of 5×10^{-3} *M* on the activity of glucose-6-phosphatase assayed by the method of Cori and Cori[15] in homogenates of rat and of rabbit liver. This lack of inhibition by tolbutamide added *in vitro* has been confirmed by other workers.[16, 17] However, a decrease in the activity of this enzyme in the livers of rats given the drug for one or two days has been observed.[11, 16] This effect develops after the initial hypoglycemia and probably does not represent a direct effect of the drug. It may be analogous to changes in activity of the enzyme seen after insulin treatment, which are presumed to be secondary to other metabolic effects and to reflect rather than to cause changes in glucose output by the liver.[18]

Glucose formation by liver slices. Sutherland[19] has shown that when slices of liver obtained from fed animals are incubated *in vitro* the rate of release of glucose into the medium is a function of the amount of active phosphorylase present in the slices during the incubation period. The concentration of the active form is dependent upon the relative rates of inactivation (dephosphorylation) and reactivation (phosphorylation) of phosphorylase. The former is catalyzed by a phosphatase,[20] while phosphorylation of the inactive dephosphophosphorylase involves the action of a phosphokinase.[21] When rabbit liver slices are incubated *in vitro* in buffer alone, the process of inactivation is predominant and phosphorylase activity decreases rapidly. If epinephrine or glucagon is present during the incubation, a higher level of active phosphorylase is maintained and is manifested in an increased formation of glucose under these conditions. This effect, which mirrors the action of epinephrine and of glucagon *in vivo*, is caused by an increase in the rate of resynthesis of active phosphorylase.[21] Because of the importance of these reactions in the regulation of glucose production by the liver it was considered that this enzyme system might be the site of action of tolbutamide. In the experiments to be described, the amount of glucose released by liver slices incubated *in vitro* under various conditions served as an indication of alterations in the activity of this system.

For each experiment successive slices from a single piece of liver were used. As shown in TABLE 1, tolbutamide had no effect on the release of glucose from rat or from rabbit liver slices. The value in parentheses is the mean of the percentage effects of tolbutamide calculated for each pair of slices, one with and one without the drug. The effect of tolbutamide on the increment in glucose release produced by glucagon was next explored. TABLE 2 contains the results of preliminary experiments employing amorphous insulin as a source of glucagon activity. In this brief series the glucagon effect was consistently lower in the slices incubated with tolbutamide. The inhibition by tolbutamide (5×10^{-3} M) of the effects of purified glucagon

TABLE 1
EFFECT OF TOLBUTAMIDE ON GLUCOSE RELEASE BY LIVER SLICES

	Tolbutamide 5×10^{-3} M	Mg glucose per gm liver	
		Mean	S.E.M.
Rat (6)	0	6.1	0.60
	+	6.1	0.45
(Tolbutamide effect)		(+2.3 per cent)	(8.8)
Rabbit (9)	0	4.7	6.48
	+	4.3	0.41
(Tolbutamide effect)		(−7.0 per cent)	(5.6)

(6 experiments) and of epinephrine (10 experiments) is shown in TABLE 3. Studies with epinephrine have been repeated many times in experiments not included in this table, with similar results. Several lower concentrations of tolbutamide have also been used. In a short series of experiments with 5×10^{-4} M tolbutamide the epinephrine effect was decreased about 40 per cent.

Although these experiments could be interpreted to indicate an action of the drug at the level of phosphorylase, it was possible that inhibition of the increased glucose output induced by epinephrine or glucagon was due to an inhibition of either phosphoglucomutase or of glucose-6-phosphatase insuffi-

TABLE 2
EFFECT OF TOLBUTAMIDE ON GLUCOSE RELEASE BY RABBIT LIVER SLICES INCUBATED WITH "GLUCAGON" (AMORPHOUS INSULIN)

Experiment	"Glucagon" effect Mg. glucose per gm. liver		Per cent effect
	Control	Tolbutamide	
1	+2.6	+1.2	−54
2	+3.3	+1.7	−49
3	+1.6	+0.7	−47
4	+3.2	+1.2	−63
Mean	+2.7	+1.2	−53

TABLE 3
EFFECT OF TOLBUTAMIDE ON GLUCOSE RELEASE BY RABBIT LIVER SLICES INCUBATED WITH GLUCAGON OR EPINEPHRINE

Tolbutamide 5×10^{-4} M	Glucagon effect*	
	Mean	S.E.M.
0..........................	2.0	0.23
+..........................	0.2	0.07
% inhibition due to tolbutamide......	87.3	3.6
	Epinephrine effect*	
0..........................	3.2	0.26
+..........................	0.6	0.22
% inhibition due to tolbutamide........	85.7	7.6

* Extra mg. glucose released per gm. of liver due to glucagon or epinephrine.

cient to alter the control rate of glucose output, but sufficient to make one of these reactions the rate-limiting step in glucose release when the phosphorylase step was accelerated. Therefore, the effect of tolbutamide on the glucose output was determined after the addition of glucose-1-phosphate to the medium. The results of these experiments are shown in FIGURE 1. The first three bars show the glucose output of the controls, the slices with epinephrine alone, and those with epinephrine plus tolbutamide. The last three bars show the glucose output with glucose-1-phosphate alone, plus epinephrine, and plus epinephrine and tolbutamide. Data not shown here indicate that tolbutamide had no effect on the output of glucose in the presence of glucose-1-phosphate alone. Even in the presence of tolbutamide the addition of glucose-1-phosphate leads to a great increase in glucose output (last bar). The tolbutamide effect noted when both epinephrine and glucose-1-phosphate are present corresponds roughly to the increment in glucose output associated with the addition of epinephrine. Thus, no effect on glucose-6-phosphatase or phosphoglucomutase need be postulated to explain the results.

It is evident that, under the conditions of these studies, tolbutamide interferes with the conversion of liver glycogen to glucose. More specifically, it may be deduced that the phosphokinase which catalyzes the formation of active phosphorylase is inhibited, as suggested previously.[14] Berthet *et al.*[17]

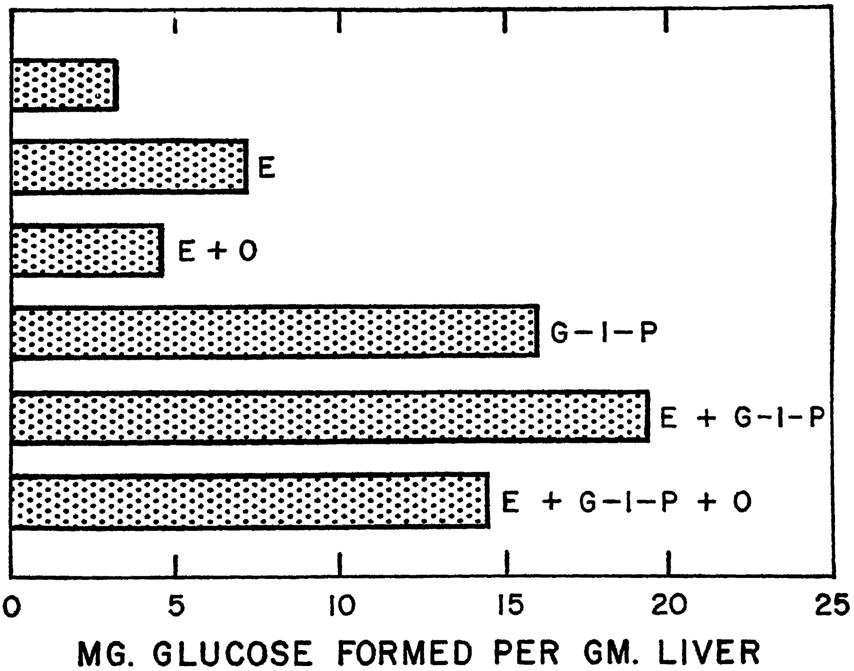

FIGURE 1. Glucose release by rabbit liver slices with additions as noted on each bar. E = epinephrine, O = tolbutamide, G-1-P = glucose-1-phosphate.

have recently reported that tolbutamide and carbutamide inhibit phosphorylase reactivation in a liver homogenate system. The concentrations used, as in the experiments reported here, were somewhat higher than the blood levels required to produce hypoglycemia. These workers also studied the effects of other sulfonyl compounds on the phosphorylase reactivation system and were unable to correlate inhibition in this system with effects on blood sugar *in vivo.* Neither the latter observation nor the fact that the concentrations of sulfonylurea needed to cause inhibition *in vitro* are five to ten times the usual blood levels excludes an effect at this site as the mechanism of hypoglycemic action since there are numerous factors, such as intracellular localization, binding to protein, and rates of metabolism and excretion, that are important in the determination of the efficacy of a drug in the intact animal.

Although it would be unwarranted to conclude that the *in vivo* effects of tolbutamide are due exclusively to the inhibition of synthesis of active phosphorylase which has been demonstrated *in vitro,* there is considerable evidence compatible with such a possibility. On the other hand, there remains a large body of data that is difficult to explain on the basis of an effect of the sulfonylureas at any single site. Some of the apparently contradictory findings may be due to the wide variations in dosage and differences in animal species employed in these studies. Furthermore, in all probability carbutamide and tolbutamide do, to a greater or lesser degree, influence more than one enzyme system, and it is quite possible that some of the effects of the two drugs are different qualitatively and quantitatively. It is likely that for a complete understanding of the mechanism of action of these drugs we must await further knowledge of the factors involved in the regulation and integration of metabolic pathways at the molecular level.

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STUDIES ON THE ABSORPTION, MECHANISM OF ACTION, AND EXCRETION OF TOLBUTAMIDE IN THE RAT

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Introduction

In a recent review Levine[1] has outlined the theories postulated for the mechanism of the hypoglycemic action of tolbutamide (Orinase*). Like similar compounds studied earlier by Loubatières[2] (sulfanilamido-isopropylthiadiazole) and Chen[3] (sulfanilamido-cyclopropylthiadiazole), tolbutamide is not an effective hypoglycemic agent in depancreatized or thoroughly alloxanized animals, as shown by Mirsky *et al.*[4] and Baender and Scholz.[5] Therefore, it appears that active islet tissue must be present if these drugs are to be fully active. In addition, Miller *et al.*[6, 7] have shown that tolbutamide increases liver glycogen only in the fasted intact rat. Work reported by others indicates that this is probably a result of decreased glycogen breakdown.[8-14]

Therefore, in the rat it seems reasonable to conclude that tolbutamide probably stimulates insulin release by the pancreas and, in the fasted animal at any rate, decreases hepatic glycogen breakdown so that the over-all mechanism involved in the hypoglycemic response differs from that of injected insulin. In a further attempt to compare responses of rats to tolbutamide with known actions of insulin, we have measured the rate of oxidation of C_6^{14}-glucose (uniformly labeled) to $C^{14}O_2$ by healthy and severely alloxan-diabetic animals. In addition, the resulting specific activity of liver and muscle glycogen was determined in healthy rats.

Tolbutamide produces hypoglycemia rapidly after oral administration to rats, dogs,[6] and humans.[15] Since these observations suggested effective gastric absorption it was of interest to study to what extent this was possible. The present report also includes studies concerning the urinary excretion of tolbutamide by the rat.

Experimental

For the absorption studies, 24-hr.-fasted male albino rats (130 to 135 gm.) were divided into 2 groups of 6 rats each. The rats were anesthetized individually with ether and surgically prepared by either ligation of the pylorus alone or ligation of both the pylorus and the duodenum at a point about 3 cm. from the pylorus. For gastric absorption studies, 1 ml. of an aqueous solution of the sodium salt of S^{35}-tolbutamide† (270 mg./kg., 7.4×10^5 c.p.m.) was injected into the lumen of the stomach using a 1 ml. capacity tuberculin syringe. For duodenal studies 0.2 ml. of solution containing the same dose of tolbutamide was injected into the ligated segment. The abdominal incision was closed with wound clips during the experimental period. Periodically the rats were anesthetized with ether, oxalated blood samples (0.3 ml. by cardiac puncture) were taken for blood sugar analysis (micro Folin-Wu[16]), and the plasma counted for S^{35} activity using corrections for self-absorption and decay. After 7 hours, the remaining S^{35} activity recovered from the absorption areas was counted and the percentage of absorption calculated.

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* The Upjohn trademark for its brand of 1-butyl-3-*p*-tolylsulfonylurea.

† Generously supplied by Farbwerke Hoechst, Frankfurt-am-Main, Germany.

For the studies on mechanism of action, 24-hr.-fasted or well-fed intact male albino rats (150 to 155 gm.) were given either 4.14×10^6 c.p.m. (0.8 mg.) or 9.95×10^6 c.p.m. (1.93 mg.) of C_6^{14}-glucose or were given 6.10×10^6 c.p.m. (0.18 mg.) of 2-C^{14}-glycine intraperitoneally. The equipment used for collecting and counting the respiratory $C^{14}O_2$ has been described, and the procedures used for determining and counting liver and gastrocnemius muscle glycogen have been published elsewhere.[7] Some rats were given tolbutamide (270 mg./kg. orally), while others were given 3 units/kg. of crystalline insulin* subcutaneously. In fasted animals the hypoglycemia produced by the injected insulin was more intense but shorter in duration than that observed from tolbutamide. In well-fed rats the maximum hypoglycemia from insulin was similar to that from tolbutamide, but the latter was longer acting: in the insulin-treated animals the hypoglycemia was absent after 3 hours, while tolbutamide was effective for from 5 to 8 hours. Under these experimental conditions, the hypoglycemic response from tolbutamide appeared to be about equal in fasted and in well-fed animals.

For the excretion studies, 6 well-fed male albino rats (about 200 gm. each) were given S^{35}-tolbutamide (270 mg./kg. orally) and were placed in individual metabolism cages. The voided urine was collected under toluene. A measure of the total S^{35} activity excreted was obtained by counting aliquots of urine dried on planchet cups directly. In order to isolate the possible urinary excretion products, the pooled urine was concentrated to dryness *in vacuo* and the residue extracted with 1:1 methanol-ethylene dichloride (25 ml. of solvent per 10 ml. of original urine) for 20 minutes. The resulting mixture was centrifuged and the clear solvent layer (containing the S^{35} activity) recovered. The extract was concentrated *in vacuo* and chromatographed by downward displacement on 613 Eaton-Dikeman paper for 15 hours in a solvent system containing 81 per cent of *n*-butanol and 19 per cent water (by weight), to which was added 2 per cent of piperidine. This solvent system was shown capable of separating tolbutamide from the major human excretion product (1-butyl-3-*p*-carboxyphenylsulfonylurea) described by Fajans *et al.*[17] The S^{35} peaks were localized on the dried paper by the use of a Forro scanner† connected to a Wheelco capacilog recorder.

The statistical significance calculated in all tables is the standard error of the mean calculated from the range.

Results and Discussion

Absorption. It was of interest that S^{35} activity was absorbed from the stomach rather rapidly; the blood plasma radioactivity was already equiva-

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[*] U-40 Iletin insulin, Eli Lilly and Company, Indianapolis, Ind

[†] Volk Radio Chemical Co., Chicago, Ill.

lent to about 60γ of tolbutamide/ml. at one-half hour after administration, with the peak plasma concentration of S^{35} activity observed after 3 hours (TABLE 1). Although these plasma figures represented only a small portion of the total dose at any single period, by the third hour the blood sugar concentrations of these rats were lowered at least 30 per cent compared to those of controls injected with saline. After 7 hours as much as 80 per cent of the original dose had been absorbed, since only 20 per cent was recovered from the stomach contents. For rats receiving the drug via the duodenal segment, the plasma concentrations of S^{35} were twice as high, and after 7 hours these animals had absorbed nearly all of the dose (TABLE 1).

Mechanism studies. There was a significant increase in the rates of oxidation of glucose by all healthy (nondiabetic) rats given either tolbutamide or insulin (TABLE 2, groups 1 to 6). For the fasted rats the increased rates observed with tolbutamide and insulin approached their maximum values (78 and 178 per cent increase respectively) at about one-half hour after the drugs were given. Increased rates were present up to the second hour. In well-fed rats the increases (32 and 70 per cent, respectively) were smaller and somewhat different in their time relationships: for example, the increased oxidation rate induced by injected insulin was no longer present at one hour, while the response from tolbutamide was more prolonged.

Alloxan diabetic rats responded well to injected insulin, showing an increased glucose oxidation rate of 126 per cent by the second hour. On the other hand, tolbutamide did not stimulate oxidation either when given alone or together with insulin (TABLE 2, groups 7 to 10). Therefore, under these experimental conditions, tolbutamide did not appear to be able to enhance the response of insulin by retarding its destruction. From these respiratory

TABLE 1
ABSORPTION OF S^{35}-TOLBUTAMIDE BY RATS
(270 mg./kg.)

Time after dose	Gastric			Duodenal		
	Plasma S^{35} activity		Absorption	Plasma S^{35} activity		Absorption
	Fraction of dose	Equiv. tolbutamide conc.		Fraction of dose	Equiv. tolbutamide conc.	
Hr.	%	γ/ml.	%	%	γ/ml.	%
½	0.8 ± 0.07	62 ± 5		1.3 ± 0.20	101 ± 15	
1	1.0 ± 0.05	77 ± 5		2.1 ± 0.21	164 ± 21	
3	1.3 ± 0.10	100 ± 7		2.6 ± 0.20	202 ± 20	
5	0.9 ± 0.05	70 ± 4		2.1 ± 0.10	164 ± 14	
7	0.8 ± 0.06	62 ± 5	81 ± 2	1.5 ± 0.08	93 ± 11	94 ± 3

TABLE 2

THE EFFECTS OF TOLBUTAMIDE AND INSULIN ON THE CUMULATIVE EXHALATION OF $C^{14}O_2$ AS PER CENT OF THE INJECTED DOSE OF C_6^{14}-GLUCOSE*

Group	Per cent injected C^{14} activity oxidized to $C^{14}O_2$ after				
	½ hr.	1 hr.	2 hr.	3 hr.	5 hr.
Healthy					
1. Fasting controls (8)†	2.3 ±0.1	5.3 ±1.4	18.7 ±3.4	25.0 ±3.7	31.8 ±3.3
2. Fasting tolbutamide (8)	4.1 ±0.4	8.9 ±1.5	17.3 ±3.1	22.5 ±5.0	27.3 ±4.9
3. Fasting insulin (10)	6.4 ±0.6	13.0 ±1.2	21.2 ±2.9	26.0 ±2.5	33.6 ±5.0
Healthy					
4. Well-fed controls (6)	5.4 ±1.5	14.7 ±1.0	24.2 ±2.9	34.1 ±2.0	42.0 ±5.7
5. Well-fed tolbutamide (4)	6.6 ±2.7	19.8 ±3.6	32.3 ±0.8	39.8 ±2.6	43.2 ±4.8
6. Well-fed insulin (5)	9.2 ±0.3	13.7 ±2.7	22.1 ±3.4	28.4 ±3.4	36.4 ±5.7
Alloxan Diabetic‡					
7. Fasting controls (4)	0.9 ±0.2	2.6 ±0.6	6.9 ±1.5	10.8 ±2.3	15.4 ±3.2
8. Fasting tolbutamide (4)	1.2 ±0.3	2.6 ±0.6	6.6 ±1.4	11.9 ±2.5	19.6 ±4.1
9. Fasting insulin (4)	1.6 ±0.3	5.2 ±1.1	15.6 ±3.3	22.7 ±4.8	29.4 ±6.2
10. Fasting tolbutamide + insulin (4)	1.6 ±0.3	5.2 ±1.1	17.5 ±3.7	26.3 ±5.5	33.4 ±7.0

* Reproduced from Miller *et al.*[7] (by courtesy of the *Journal of Pharmacology and Experimental Therapeutics*).

† Numbers of rats used to obtain group mean.

‡ The 24-hr. fasting blood sugars for the 4 alloxan diabetic rats were 385, 420, 395, and 360 mg. per cent. These rats were diabetic for about 8 months prior to use. The same 4 alloxanized animals were used in each experiment.

data, it is concluded that the increased capacity of healthy rats to oxidize glucose was a result of a stimulation of the insulin secretion mechanisms by tolbutamide.

If tolbutamide can stimulate the release of insulin from the pancreas, then under appropriate experimental conditions this drug should encourage the synthesis of muscle glycogen; indeed, this was the case. The requirements for demonstrating the incorporation of C_6^{14}-glucose into muscle glycogen were found to be those of fasting the animal and providing sufficient C_6^{14}-glucose. For example, after 5 hours both tolbutamide and the injected insulin failed to incorporate glucose into muscle glycogen in well-fed rats given only 0.8 mg. of C_6^{14}-glucose (TABLE 3, groups 4 to 6). In fasted animals given the small dose of radioglucose only the parenteral insulin was effective, but when the C_6^{14}-glucose was increased to 1.93 mg. per fasted rat, both tolbutamide and injected insulin produced increased specific activity in the muscle glycogen (TABLE 3, groups 7 to 9).

Although the hypoglycemic action of tolbutamide is probably due primarily to its "insulin-secretory" action, the drug, as mentioned earlier, can apparently add to the hypoglycemic response by decreasing the rate of glycogen breakdown in the liver. It is important to point out that in our experiments only fasted rats given tolbutamide showed increased liver glycogen content and increased specific activity from C^{14} incorporation (TABLE 3, groups 1 to 3, 7 to 12). Hence, in the intact well-fed rat tolbutamide can produce hypoglycemia and concomitant increased oxidation of glucose without apparently altering liver glycogen.

Urinary excretion of tolbutamide. Approximately 72 hours elapsed before rat urine samples were free of S^{35} activity; most of the activity (about 50 per cent of the dose) was excreted during the first 24 hours. The chromatographic separation of the S^{35} activity is demonstrated in FIGURE 1. There can be little doubt that *A*, the primary excretion product found, was not tolbutamide, *O*; neither was it identical with the human transformation product mentioned earlier, which has the same R_f as product *C*. Product *B* was different from all the compounds mentioned and was present to about the same extent as product *C*. The concentrations of *B* and *C* were only about one tenth that of product *A*. Chemical characterization of these excretion products is now in progress. Preliminary evidence from ultraviolet and infrared analysis indicates that product *A* is probably a conjugate of tolbutamide with some organic base.

TABLE 3

THE EFFECTS OF TOLBUTAMIDE AND INSULIN ON LIVER AND MUSCLE GLYCOGEN CONTENT OF HEALTHY RATS GIVEN TRACER DOSES OF C_6^{14}-GLUCOSE OR 2-C^{14}-GLYCINE WITH CORRESPONDING SPECIFIC ACTIVITY MEASUREMENTS*

Group†	Liver glycogen: Content	Liver glycogen: Specific activity	Liver glycogen: Approx. per cent of C^{14} dose incorporated	Muscle glycogen: Content	Muscle glycogen: Specific activity	Muscle glycogen: Approx per cent of C^{14} dose incorporated
	mg. %	c.p.m. mg.	%	mg. %	c.p.m. mg.	%
4.14 × 10^6 c.p.m. C_6^{14}-glucose (0.8 mg.) per rat						
1. Fasting controls (8).	240 ± 43	1348 ± 44	<1	322 ± 28	0	0
2. Fasting tolbutamide (8)	1100 ± 198	3300 ± 200	4	294 ± 22	trace	0
3. Fasting insulin (10)	235 ± 86	1465 ± 61	<1	284 ± 65	1690 ± 400	9
4. Well-fed controls (6).....	2880 ± 290	0	0	377 ± 9	0	0
5. Well-fed tolbutamide (4).	3103 ± 298	0	0	398 ± 89	0	0
6. Well-fed insulin (5)	2430 ± 164	0	0	461 ± 30	0	0
9.95 × 10^6 c.p.m. C_6^{14}-glucose (1.93 mg.) per rat						
7. Fasting controls (6).	284 ± 46	2200 ± 35	<1	327 ± 10	0	0
8. Fasting tolbutamide (6)...	912 ± 18	5300 ± 175	3	300 ± 15	1850 ± 300	4
9. Fasting insulin (6)......	330 ± 34	2010 ± 43	<1	340 ± 25	2500 ± 75	8
6.10 × 10^6 c.p.m. 2-C^{14}-glycine (0 18 mg.) per rat						
10. Fasting controls (2).......	92 ± 8	0	0	385 ± 125	0	0
11. Fasting tolbutamide (2)...	960 ± 250	1300 ± 150	1	323 ± 210	0	0
12. Fasting insulin (2).......	164 ± 60	0	0	320 ± 170	0	0

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* Reproduced from Miller *et al.*[7] (by courtesy of the *Journal of Pharmacology and Experimental Therapeutics*).

† The first 6 groups are the same rats used for the corresponding respiratory studies shown in TABLES 2 and 3. Special groups 7 through 9 were given higher doses of C_6^{14}-glucose than were used for respiratory studies, in an effort to increase the specific activity of the isolated glycogen; CO_2 measurements were not made on these rats. The same doses of tolbutamide and insulin were used in all animals, however.

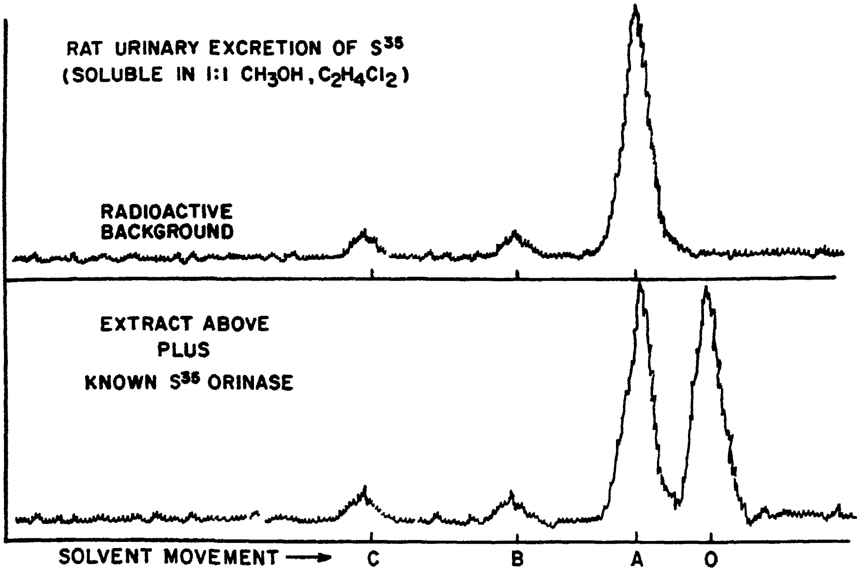

FIGURE 1. Paper chromatographic separation of radioactive urinary excretion products from rats given S^{35}-tolbutamide orally, as recorded by a paper strip monitor. Known S^{35}-tolbutamide was added directly to an aliquot of the same urine containing the excretion products and extracted in their presence. (A) R_f 0.79, primary rat excretion product; (B) R_f 0.64, an additional excretion product; (C) R_f 0.46, another excretion product, perhaps identical with the primary human excretion product (1-butyl-3-*p*-carboxyphenylsulfonylurea); (O) R_f 0.87, tolbutamide (1-butyl-3-*p*-tolylsulfonylurea).

Summary

(1) Both tolbutamide (Orinase) and injected insulin increased the rate of oxidation of C_6^{14}-glucose by nondiabetic rats. These agents also increased the specific activity of gastrocnemius muscle glycogen isolated from 24-hour-fasted nondiabetic rats given a "high-activity" tracer dose of glucose.

(2) Parenteral insulin stimulated the oxidation of glucose by severe alloxan diabetic rats, while tolbutamide was without effect either alone or in the presence of injected insulin.

(3) Since in the intact well-fed rat tolbutamide produced hypoglycemia and increased glucose oxidation, but had no apparent effect on liver glycogen, it is believed that the increased liver glycogen observed in fasting rats is incidental to the "insulinlike" ability of tolbutamide to stimulate glucose utilization.

(4) It is concluded that tolbutamide produces hypoglycemia mainly by its influence on pancreatic tissue capable of insulin synthesis to yield more insulin.

(5) Tolbutamide was absorbed from the stomach as well as the duodenum, and is excreted in the urine as a conjugate different from the major human excretion product.

Acknowledgment

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PERFUSION STUDIES WITH SULFONYLUREAS IN DOGS*

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These studies were undertaken to determine the site of hypoglycemic action of the available sulfonylureas. In a preliminary report of acute perfusion experiments[1] we suggested a pancreatic site of action.

Our results now come from thirty-four experiments in which the compounds and control solutions have been injected at various locations and changes in blood glucose observed. The experiments have been grouped and the data averaged, permitting an improved measure of differences of effect by various injection routes. Statistical validation still is not possible, however, because of the small size of each group.

Methods

Small amounts of sodium tolbutamide (Orinase Sodium†) or carbutamide‡ were perfused through the pancreas or liver or infused into a peripheral vein of normal dogs under Nembutal anesthesia. Injections were given with a constant infusion pump through small polyethylene catheters tied into the vessels used. As shown in FIGURE 1, pancreatic perfusions were made through the stump of the right gastroepiploic branch of the gastroduodenal artery, thus permitting perfusion of the test solutions without interrupting the blood flow to the pancreas. When comparable injections were given into a peripheral vein, anesthesia and laparotomy with exposure of the pancreaticoduodenal artery were done as usual in order to reproduce all conditions of the pancreatic experiments except the arterial infusion itself.

Perfusions of the liver were made through the left hepatic artery in some experiments and through a branch of the portal vein in others. Cannulation of the hepatic artery interrupted the blood flow in one of the two branches (FIGURE 1), but the rest of the blood supply to the liver remained intact. A control solution lacking sulfonamide (blank) was perfused through the pancreas and liver.

Screening experiments were done, using longer injection periods and larger and smaller sulfonamide dosages than those reported here. In all of the present experiments, however, injection periods were 20 min. and sulfonamide dosages, 7 mg. per kg. of body weight, or a total dose of about 80 to 160 mg. per dog. Injection rates were about 0.5 ml. per min., and the *p*H of all solutions was 7.2.

Blood glucose was determined by the Somogyi-Nelson method 2 or 3 times before injection and at intervals of 10 to 30 min. for 4 hr. after injection.

* The investigation on which this paper is based was supported by grants and materials from The Upjohn Company, Kalamazoo, Mich., and from Eli Lilly and Company, Indianapolis, Ind.

† Sodium salt of Orinase, The Upjohn Company's preparation of 1-butyl-3-*p*-tolylsulfonylurea or tolbutamide.

‡ 1-Butyl-3-sulfanilylurea or BZ-55, supplied by Eli Lilly and Company.

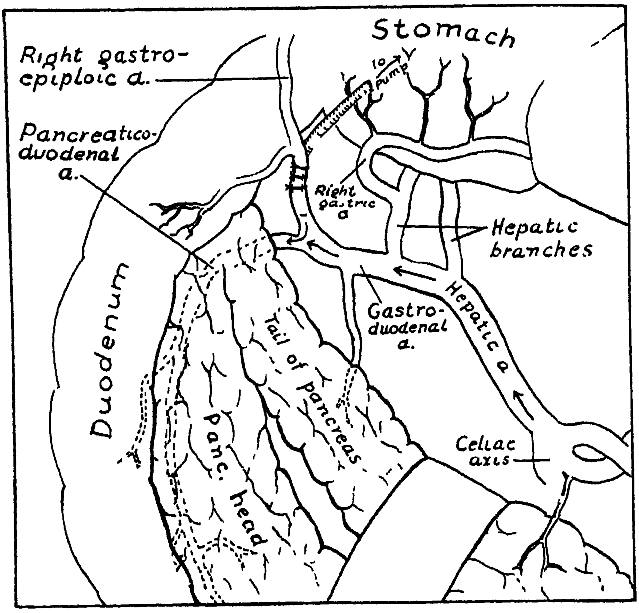

FIGURE 1. Site of injection into the gastroepiploic branch of the gastroduodenal artery. Pancreatic blood flow is not interrupted. The left hepatic artery is shown without cannula insertion. It was the site of some of the hepatic perfusions.

Individual values were calculated in percentage over or under the preoperative value in all experiments, and the data for all dogs in each experimental group were averaged at each of the various time intervals. Composite curves were then constructed for each substance injected and each route of injection. As in all experiments of this type, different animals showed a range of blood glucose values, but averaging of results yielded, as a rule, smooth composite curves.

Results

In FIGURE 2 the average results of pancreatic perfusion of tolbutamide in 6 dogs are compared with those of carbutamide in 4 others. Blood glucose values were comparable in both sets of experiments until an hour or two after the start of injection, when mild hypoglycemia began; this was more pronounced after tolbutamide than after carbutamide. Peripheral blood sulfonamide concentrations with carbutamide were 2.5 to 3.0 mg. per 100 ml., about one fifth of those seen after oral administration of therapeutic amounts to normal subjects. We were unable to measure blood tolbutamide satisfactorily and can only assume that it was comparable to blood carbutamide, since dosages and infusion rates were identical.

Whether the difference in response to the two compounds was due to injection of tolbutamide as its sodium salt or whether there was greater sensitivity to tolbutamide than to carbutamide is not clear.

Average values for two control experiments in which a blank solution was

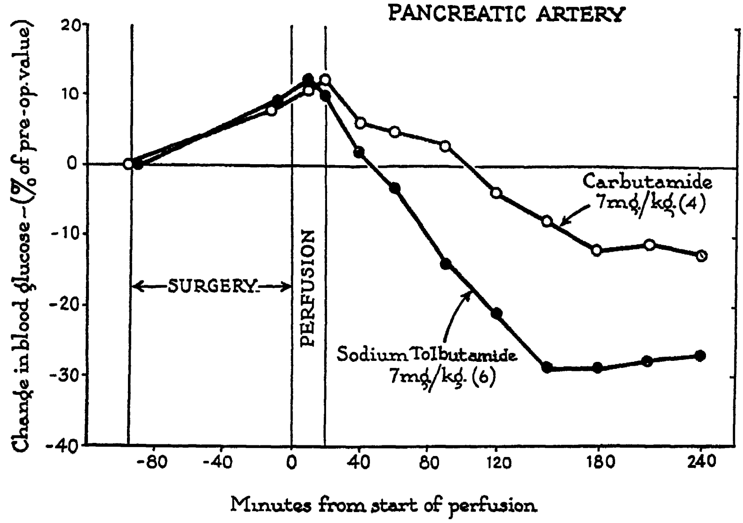

FIGURE 2. Mean curves of blood glucose after intrapancreatic perfusion of sodium tolbutamide (6 dogs) and carbutamide (4 dogs). Drug dosages and injection rates were the same.

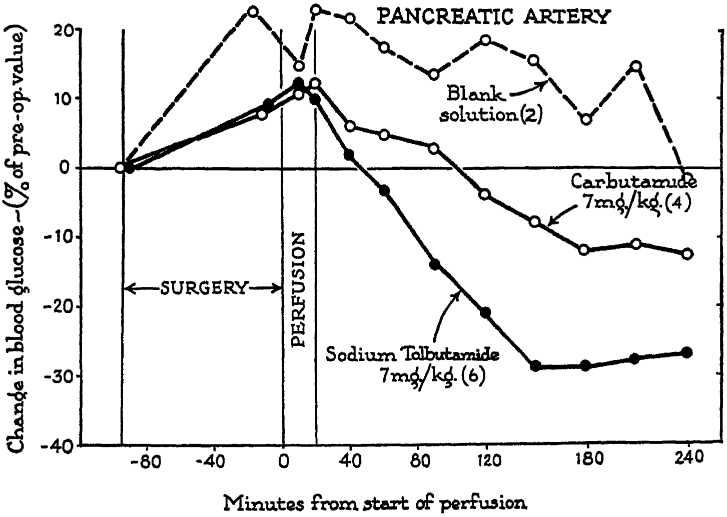

FIGURE 3. Mean curves of blood glucose after perfusion of blank solution through pancreas (2 dogs) compared with tolbutamide and carbutamide results shown in FIGURE 2.

perfused through the pancreas are shown in FIGURE 3, in comparison with the data on tolbutamide and carbutamide. The solution was identical in composition and amount with that used before, except that the sulfonamide was omitted. The blood glucose fell approximately to its preoperative level 4 hours after the start of injection, but no hypoglycemia was seen. This makes the hypoglycemia after drug administration appear more significant.

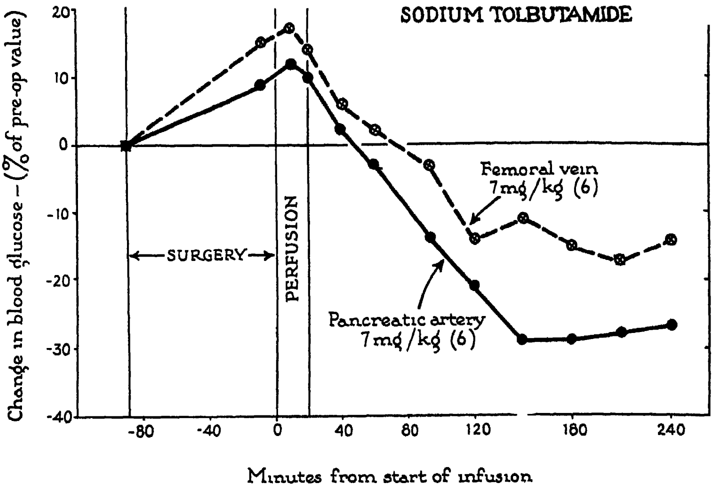

FIGURE 4. Mean curves of blood glucose after intrapancreatic (6 dogs) and femoral vein (6 dogs) injection of sodium tolbutamide. Drug dosages and injection rates were the same.

In FIGURE 4 are compared the results of pancreatic perfusion of tolbutamide in 6 dogs with those of femoral vein infusion of the same substance in 6 others. These experiments were intended to answer the important question whether hypoglycemia was greater after direct pancreatic injection than after injection peripherally. The blood glucose rose 10 to 15 per cent during anesthesia and laparotomy, and started falling at the end of the injection period. The lowest values were reached 2½ to 3 hr. later. Compared with preoperative blood glucose, the intrapancreatic route of administration led to about twice as much hypoglycemia as did the femoral vein route. Even though early blood glucose values were a little higher in the peripheral vein experiments, the difference at 2 to 4 hr. appears significant.

Since pancreatic venous blood next traverses the liver, it was conceivable that the greater hypoglycemia after intrapancreatic administration of tolbutamide was due to hepatic rather than pancreatic action. Hepatic artery and portal vein perfusions were conducted, therefore, under conditions identical with those of the pancreatic and peripheral injections. FIGURE 5 shows the effects of hepatic artery perfusion of tolbutamide in 3 dogs. A small operative increase followed by a modest reduction in blood glucose was seen, roughly comparable with the results of peripheral vein infusion. The average response to a blank control solution in 2 dogs is shown by the broken line. In contrast to all other control experiments with blank solutions, there was a small and transient fall in blood glucose not very different from that after tolbutamide.

In FIGURE 6 is shown the average response to injection of carbutamide into the portal vein in 3 dogs, in comparison with 1 control experiment with the blank solution alone. The elevation of blood glucose during anesthesia and surgery was of much greater magnitude than in any other experiment.

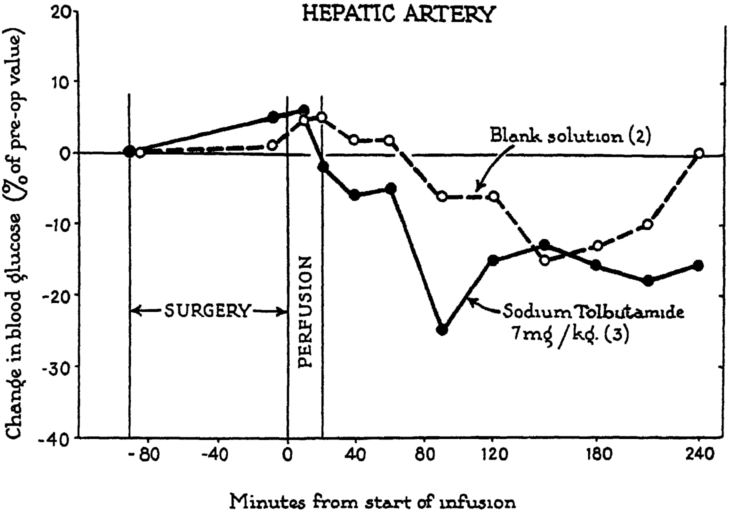

FIGURE 5. Mean curves of blood glucose after left hepatic artery perfusion of a sodium tolbutamide solution (3 dogs) and of a blank control solution (2 dogs).

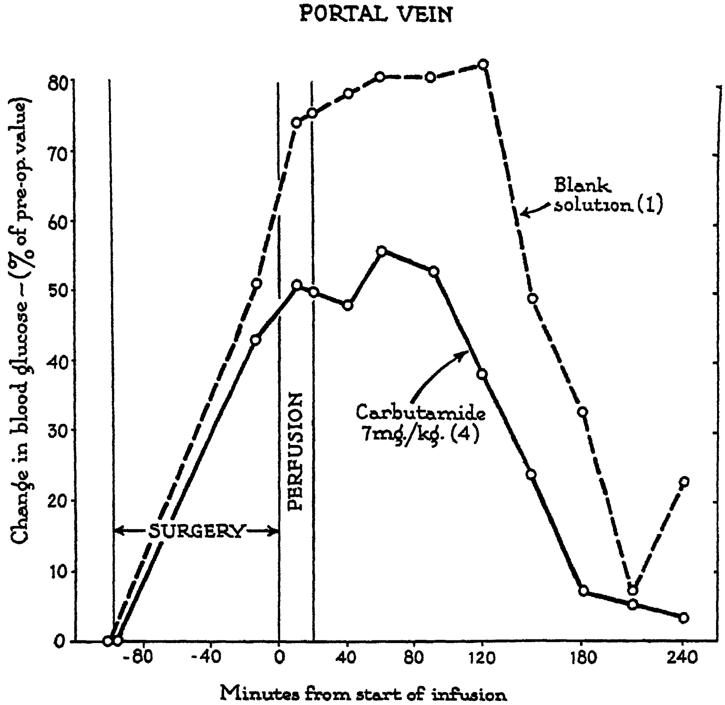

FIGURE 6. Mean curve of blood glucose after perfusion of carbutamide through liver via the portal vein (3 dogs) compared with blank solution (1 dog).

About 3 hr. after injection it fell as before, but only to the preoperative level. One experiment with the blank solution led to even greater hyperglycemia than the average from carbutamide.

No portal vein experiments were done with tolbutamide. While it is conceivable that its effect might vary from that of carbutamide, we believe that the drugs are similar enough in all other respects to make this possibility remote.

Discussion

Lacking data susceptible to statistical analysis, we are reluctant to draw firm conclusions from these experiments. We may not find it feasible to extend our study to include more than the 3 to 6 dogs per group. In that event, interpretations must remain presumptive rather than conclusive.

For the present, however, we continue to believe in a pancreatic rather than a hepatic mechanism. It is difficult for us to conceive of any hepatic mechanism for sulfonylurea hypoglycemia when direct exposure of the liver to the drug via the portal vein leads only to hyperglycemia within the 4 hours of study. Furthermore, hepatic artery perfusion of tolbutamide leads only to about the same fall in blood glucose as an inert solution given by the same route or the same drug injected into a peripheral vein. Within the limits of our experimental conditions, the conclusion that the liver plays no primary role seems fully justified.

Our evidence in favor of a pancreatic action is less convincing. Even though blood glucose levels were lower after perfusion of the drug through the pancreas, the same dosages given peripherally and into the hepatic artery did cause some hypoglycemia. Only by demonstrating hypoglycemia with pancreatic dosages too small to be effective when given by other routes can positive proof be claimed for pancreatic action by this method of study. We have done a few experiments using 1 and 3 instead of 7 mg. per kg. intrapancreatically for 20 minutes, with equivocal results.

Summary

(1) Thirty-four normal anesthetized dogs were perfused with dilute solutions of carbutamide and tolbutamide into the pancreaticoduodenal and hepatic arteries and into the portal and femoral veins. Dosages and injection rates were uniform. Blood glucose values were observed for 4 hours after the start of injection.

(2) Hypoglycemia was greatest after tolbutamide intrapancreatically. Smaller decreases in blood glucose followed introduction of tolbutamide into a hepatic artery and a femoral vein. Carbutamide intrapancreatically led to less hypoglycemia than tolbutamide, and the same compound given into the portal vein caused no hypoglycemia.

(3) These results argue strongly against a hepatic mechanism and support the concept of a pancreatic site of action for sulfonamide hypoglycemia.

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STUDIES ON THE DISPOSITION OF ISOTOPIC GLUCOSE *IN VIVO* AND *IN VITRO* UNDER THE INFLUENCE OF SULFONYLUREAS[*]

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Insulin and sulfonylureas have the common property of being able to reduce the concentration of blood glucose in normal animals. Whether or not this fall in blood glucose is elicited by the same mechanism is the current subject of discussion. As an approach to this problem, we have compared the effects of insulin and tolbutamide[§] on various aspects of glucose disposition in unanesthetized dogs and rats and in isolated tissue preparations.

Hepatic Glucose-6-Phosphatase

Recent studies from several laboratories have indicated that changes in hepatic glucose-6-phosphatase activity are associated with certain abnormalities in carbohydrate metabolism.[1, 2, 3, 4] Furthermore, it has been demonstrated that the administration of hormones to animals will alter the activity of this enzyme.[5, 6] Since glucose-6-phosphatase is the immediate enzyme involved in hepatic glucose production, the effect of insulin and tolbutamide on the activity of this enzyme has been compared. The administration of insulin and tolbutamide to normal rats over a period of 48 hr. results in a decrease in liver glucose-6-phosphatase activity to 60 to 70 per cent of normal.[7] Similar results have also been obtained by Hawkins and Haist[8] and by Tyberghein *et al.*[9] However, it was observed that within 3 hr. neither insulin nor tolbutamide diminished glucose-6-phosphatase activity, although the blood sugar was markedly decreased.

When added to liver preparations *in vitro*, tolbutamide has been shown to decrease glucose production, decrease the rate of glycogenolysis, and inhibit glucose-6-phosphatase.[7, 9, 10, 11] However, the concentration of tolbutamide required to produce these effects *in vitro* is in excess of the concentration required to produce *in vivo* effects.

Effect of Insulin and Tolbutamide on Isolated Rat Diaphragm

Insulin readily accelerated glucose utilization by isolated rat diaphragm when administered *in vitro*. Addition of tolbutamide to isolated diaphragm did not elicit such a response. However, we were interested in ascertaining whether the presence of tolbutamide in the system could augment the effect

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[†] Post-Doctoral Fellow in Medical Sciences of the National Research Council (Rockefeller Foundation for Medical Research).

[‡] Harvey Cushing Surgical Fellow. Harvard Medical School, Boston, Mass.

[§] 1-Butyl-3-*p*-tolylsulfonylurea (Orinase, The Upjohn Company, Kalamazoo, Mich.)

TABLE 1
RAT HEMIDIAPHRAGMS INCUBATED 90 MIN IN RINGER BICARBONATE MEDIUM WITH 10 MICROMOLES/ML OF GLUCOSE VALUES EXPRESSED AS MICROMOLES OF GLUCOSE DISAPPEARING FROM THE MEDIUM PER 100 MG DIAPHRAGM DRY WEIGHT

Insulin (milliunits ml)	Animals	Insulin added	Control	Per cent change with S D
25	4	28.0	15.2	+52 (±19)
2.5	4	28.5	19.6	+51 (±35)
0.25	5	15.0	14.1	+4 (±18)

of a subthreshold level of insulin on the isolated tissue.[12] Glucose utilization by rat diaphragm muscle was measured in a series of experiments with varying concentrations of insulin. The results of such experiments are given in TABLE 1. When tolbutamide was added to preparations containing insulin, no augmentation of the insulin effect was observed (TABLE 2).

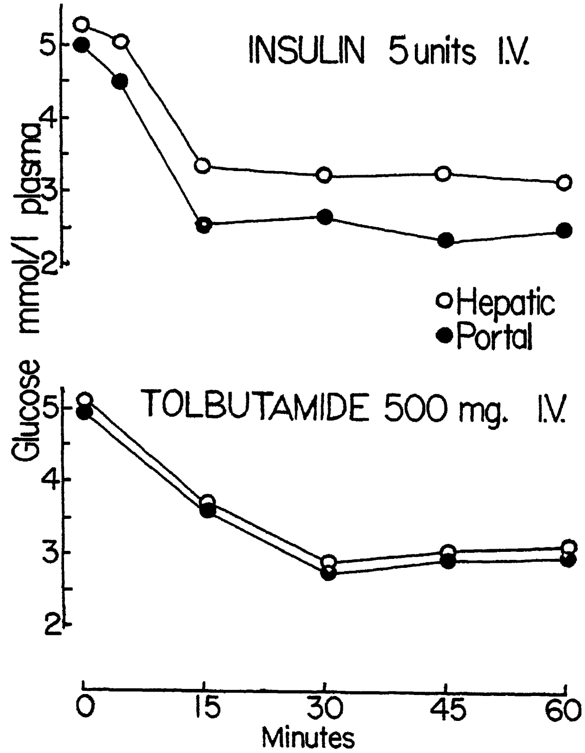

FIGURE 1. Changes in hepatic and portal glucose (Dog 3) are plotted as a function of time following injection of 5 units of glucagon-free insulin (top curves) and 500 mg. sodium tolbutamide (lower curves). Dog 3 represents a typical experiment in a series of 4 animals studied.

TABLE 2

RAT HEMIDIAPHRAGMS INCUBATED IN THE PRESENCE OF INSULIN AND ONE WITH 50 MG. PER CENT SULFONYLUREA. VALUES EXPRESSED AS μMOLES GLUCOSE DISAPPEARING FROM THE MEDIUM PER 100 MG. DIAPHRAGM DRY WEIGHT

Insulin (milliunits/ml.)	Animals	Insulin plus sulfonylurea	Insulin alone	Per cent change with S. D.
25	3	22.5	22.2	+1 (±10)
2.5	6	21.7	22.7	−3 (±15)
1.0	3	17.6	18.0	−3 (±15)
0.25	6	19.2	18.3	+8 (±26)

Portal-Hepatic Sugars in Unanesthetized Dogs

The effects of insulin and tolbutamide have been compared in intact unanesthetized dogs (20 to 21 kg.) in which polyethylene catheters had been placed in the portal vein, hepatic vein, and splenic artery. Experiments were begun on the third to fourth day following operation and were done in the following order: on the first experimental day, a glucose load was given portally and the hepatic, portal, and arterial samples withdrawn simultane-

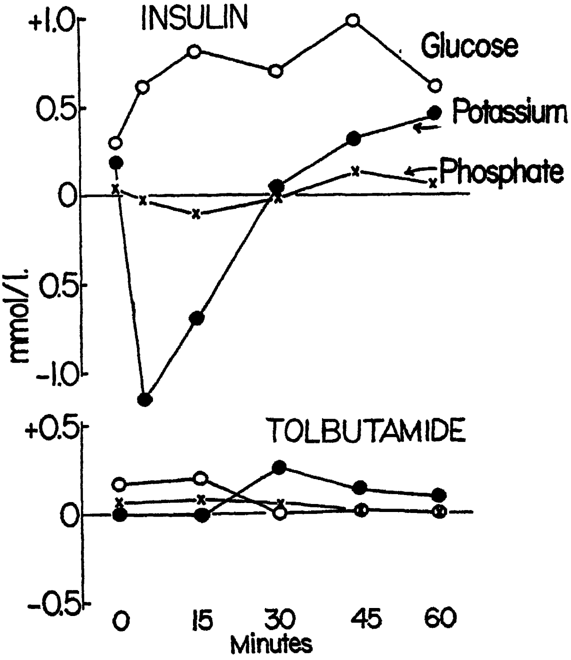

FIGURE 2. Hepatic-portal plasma differences in glucose (○), potassium (●), and phosphate (x) are plotted following injection of 5 units of insulin (top curves) and 500 mg. sodium tolbutamide (lower curves). These data were obtained on Dog 3 and represent a typical experiment in a series of 4 animals studied.

ously at 5, 15, 30, 45, and 60 min. The cells were immediately separated and the plasma analyzed for glucose, K, and PO_4. The glucose tolerance was found to be normal in all cases. On the second day, glucagon was injected via the portal catheter, and the study was repeated. These tests were performed to ascertain that the catheters were in place and operating satisfactorily. On the third day, 7 units of glucagon-free insulin (Lilly) were given intravenously and the experiment was repeated. On the fourth day, 500 mg. of Orinase Sodium (Upjohn) was injected and the experiment again repeated.

The effects of insulin and tolbutamide are compared in FIGURE 1. Dog 3 represents a typical experiment in a series of 4 animals studied. Both substances caused a rapid fall in portal and hepatic glucose, and comparable degrees of hypoglycemia were obtained in all experiments. It is observed that in the case of hypoglycemia produced by insulin the hepatic glucose levels were always appreciably higher than portal values, but following tolbutamide there was a corresponding fall in both portal and hepatic glucose.

Portal-hepatic differences have been plotted in FIGURE 2 as a function of time following insulin (top curves) and tolbutamide (lower curves). After insulin the liver appears to release glucose into the hepatic blood; K is taken up initially and then released as hypoglycemia persists; inorganic phosphate is initially taken up by the liver, then released. With tolbutamide, no significant portal-hepatic differences in glucose, K, or phosphate were observed.

In FIGURE 3, portal glucose concentration has been plotted as a function of

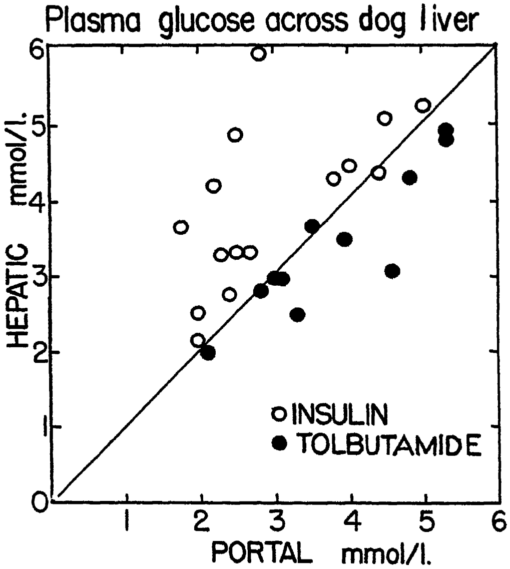

FIGURE 3. Hepatic plasma glucose in mmoles/l. has been plotted against portal plasma glucose. These points were obtained by withdrawal of simultaneous portal and hepatic blood samples in a series of 4 dogs with portal and hepatic vein cathethers. Samples obtained after insulin injection (○); after tolbutamide (●).

the hepatic glucose concentration. These points represent all analyses done on a series of 4 dogs. The line at 45 degrees indicates equal glucose concentration in portal and hepatic plasma. All points above this line represent concentration of hepatic glucose greater than portal glucose; all points below the line represent higher portal than hepatic glucose. Insulin hypoglycemia (open circles) appears to result in release of hepatic glucose. However, there was no evidence of hepatic glucose production after tolbutamide (closed circles). Similar results on the effect of tolbutamide on portal and hepatic glucose have been reported previously by Purnell *et al.*[14]

Disposition of Blood Glucose in Rats

To extend these studies we have followed in rats the fall in specific activity of blood glucose tagged by the intravenous injection of 1 mg. (1.8 μc.) glucose-C^{14}. When log specific activity of blood glucose was plotted as a function of time in fed normal rats (FIGURE 4) injected with saline and glucose, a typical linear decay curve was obtained. When insulin (Lilly glucagon-free, 0.25 units) and glucose were injected, a more rapid rate of decay was observed. The fall in specific activity of blood glucose from the liver probably results from a dilution with unlabeled glucose. When sodium tolbutamide (Upjohn, 20 mg.) was injected, the fall in specific activity of blood glucose was identical with that of the saline-injected controls. The same degree of hypoglycemia was produced by insulin and tolbutamide.

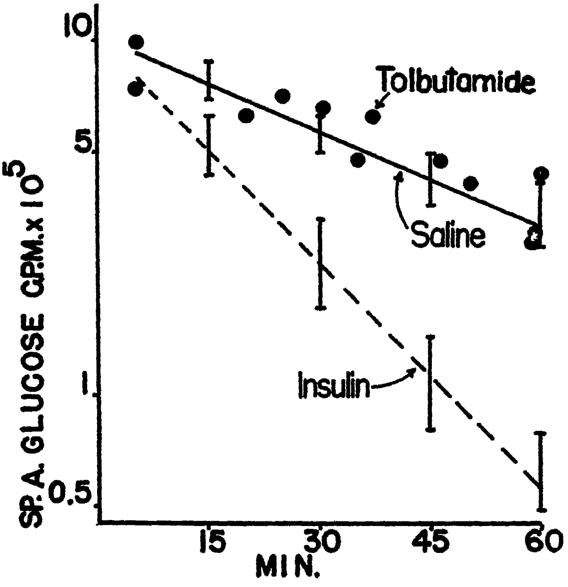

FIGURE 4. Log specific activity of blood glucose has been plotted as a function of time in variously treated fed normal rats after injection of 1 mg. (1.8 μc.) glucose-C^{14}. Each curve represents the mean of 3 experiments. Total spread of points is indicated (I). Individual points following tolbutamide injection (●) have been plotted.

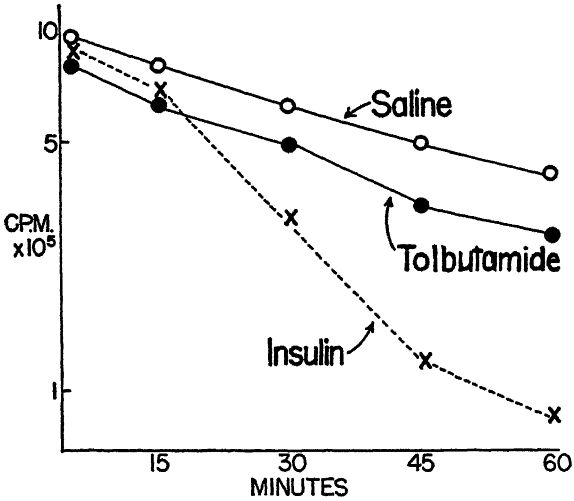

FIGURE 5. Changes in specific activity of blood glucose after injection of saline (○), 20 mg. of tolbutamide (●), and 0.25 units of insulin (x) to fasted rats are shown. Conditions are identical with those given in FIGURE 4.

Cori and Cori[14] have demonstrated that with insulin hypoglycemia, epinephrine is released from the adrenal and liver glycogen is broken down. We had reasoned that, if this were the case, dilution of blood glucose could be minimized by using fasting rats. The experiments were therefore repeated in animals that had been fasted for 24 hours prior to the injection. The results of such an experiment are given in FIGURE 5. Once again, insulin caused a greater dilution than that obtained with tolbutamide or saline. As in the previous experiments, the same degree of hypoglycemia was produced by both insulin and tolbutamide.

Effect of Insulin and Tolbutamide on Liver and Muscle Glycogen

Male rats, 220 to 250 gm., were fasted for 24 hr. and injected intravenously with 1 mg. (1.8 μc.) glucose-C^{14}, together with either saline, insulin (0.25 units), or tolbutamide (20 mg.). At the end of 1 hr. the animals were killed and exsanguinated, and liver and muscle samples were rapidly removed and placed in hot 30 per cent KOH. Tissue glycogen was isolated and hydrolyzed to glucose,[15] which was determined colorimetrically[16] and assayed for C^{14} as the phenyl glucosazone.[17] The results of these experiments are given in TABLE 3. The animals injected with insulin had deposited very little of the C^{14} from the injected glucose as liver glycogen. The animals injected with tolbutamide, however, deposited as much C^{14} as, if not significantly more than, the saline-injected controls. In the case of muscle, insulin resulted in appreciably more deposition of C^{14} into glycogen than did tolbutamide. Again, both insulin and tolbutamide had produced the same degree of hypoglycemia.

Although the same degree of hypoglycemia was produced in 60 minutes,

TABLE 3

ACTION OF INSULIN AND TOLBUTAMIDE ON THE INCORPORATION OF C^{14}-GLUCOSE INTO LIVER AND MUSCLE GLYCOGEN IN FASTED NORMAL RATS

Injection	Liver glycogen		Muscle glycogen	
	µM gm	c.p.m gm	µM gm.	c p.m. gm
Saline	13	1450	34	19
	3	585	18	19
Insulin	3	9	44	320
	9	25	11	242
Tolbutamide	15	2100	12	52
	6	2200	16	43

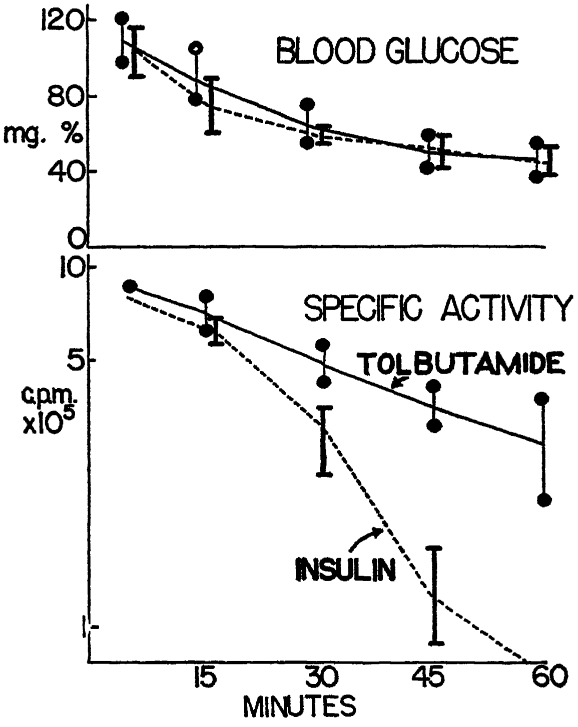

FIGURE 6 Changes in concentration of blood glucose (top curves) and specific activity of blood glucose (lower curves) after tolbutamide (intravenous) and insulin (subcutaneous) administration to fed normal rats. Each curve represents the mean of results from 3 animals, and the total spread of points is indicated.

TABLE 4
ACTION OF INSULIN AND TOLBUTAMIDE ON THE INCORPORATION OF C^{14}-GLUCOSE INTO LIVER AND MUSCLE GLYCOGEN IN FED NORMAL RATS

Injection	Liver glycogen		Muscle glycogen	
	μM/gm	c.p.m. gm.*	μM/gm	c.p.m./gm *
Saline (4)	191	100 ± 33	19	29 ± 13
Insulin (5)	88	14 ± 6.3	19	262 ± 59
Tolbutamide (4)	145	143 ± 58	18	61 ± 18

* ±S.E.

there was a marked difference in the rate of fall of blood glucose produced by insulin and tolbutamide. After intravenous injection of insulin there is, of course, an immediate drop in blood glucose, and hypoglycemia continues for the balance of the 60-minute period. With tolbutamide, the glucose concentration falls slowly over the experimental period. In order to obviate effects that might result from differences in the rate of fall of blood glucose, we compared the effects of insulin administered subcutaneously with those of tolbutamide injected intravenously; under these conditions, the same rate of blood glucose change was produced by both compounds (FIGURE 6). Changes in specific activity of blood glucose in these animals are recorded in the lower curves of FIGURE 6. As in the previous experiments, a more rapid decay of glucose specific activity was observed with insulin than with saline or tolbutamide, again indicating a more rapid dilution of the labeled blood glucose.

Liver and muscle samples were removed at the end of the experiment, and total glycogen and C^{14} content of the glycogen were determined. Insulin resulted in little incorporation of the C^{14} from the injected glucose into liver glycogen, but increased incorporation of it into muscle glycogen (TABLE 4). Although blood glucose fell to the same level with tolbutamide as with insulin, tolbutamide did not significantly increase incorporation of C^{14} from blood glucose into muscle glycogen.

In these animals liver and peripheral fat were also assayed for C^{14} content. Mean values, expressed as counts per min. per gm. of fatty acid, are

TABLE 5
ACTION OF INSULIN AND TOLBUTAMIDE ON THE INCORPORATION OF C^{14}-GLUCOSE INTO LIVER AND PERIPHERAL FATTY ACIDS IN FED NORMAL RATS
All values expressed as c.p.m./gm. fatty acid

	Liver	Peripheral
Saline (4)	300	375
Insulin (5)	940	1770
Tolbutamide (4)	1770	475

given in TABLE 5. Both insulin and tolbutamide increased the activity of liver fatty acids, but only insulin increased the activity of peripheral fatty acids.

Summary

Studies *in vitro* on the action of tolbutamide indicate that this compound neither stimulates glucose utilization by muscle nor augments the effect of insulin on muscle. However, effects that have been obtained *in vitro* would indicate that tolbutamide can decrease hepatic glucose production.

In experiments with intact dogs and rats it appears that, following insulin-induced hypoglycemia, hepatic glucose production is increased. When hypoglycemia is produced by tolbutamide injection, this does not appear to occur. Since the phenomenon of glycogenolysis following insulin hypoglycemia is believed to be mediated by the adrenal medulla, the possibility exists that tolbutamide in some way prevents normal hepatic response to epinephrine. Such a conclusion is further strengthened by observations that tolbutamide will reduce the rate of glycogenolysis in liver slices stimulated by epinephrine.[10, 11]

Insulin and tolbutamide also differ in their ability to stimulate peripheral glycogen deposition and lipogenesis. When quantities of insulin and tolbutamide that produce identical blood glucose changes were compared, insulin markedly stimulated incorporation of C^{14} from labeled glucose into muscle glycogen and peripheral fatty acids. Tolbutamide did not cause a significant increase in the C^{14} content of either glycogen or fatty acids.

At the present time we conclude that, although both insulin and tolbutamide will produce comparable degrees of hypoglycemia, there is a marked difference in the disposition of blood glucose following their administration.

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FUNCTIONAL AND HISTOLOGICAL STUDIES CONCERNING THE ACTION OF SULFONYLUREAS*

By Bruno W. Volk, Martin G. Goldner, Shirley Weisenfeld, and Sydney S. Lazarus

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Although it is established that certain sulfonylureas lower the blood sugar level, their mode of action has not yet been clarified. Several hypotheses have been advanced to explain the hypoglycemic effect. Originally Janbon and his co-workers[1] and Loubatières,[2, 3] on the basis of evidence from totally and partially pancreatectomized animals as compared to normal animals, suggested that the hypoglycemic action of sulfonylurea derivatives is based on the release of insulin from the pancreatic β cells. Other hypotheses included: an α-cytotoxic action or interference with glucagon activity;[4, 5] enhancement of insulin action by inhibition of insulinase;[6, 7] interference with hepatic glycogenolysis, either at the level of the phosphohexokinase reaction[8] or at some other step of glycogen breakdown in the liver;[9] and enhancement of the action of insulin in increasing peripheral glucose utilization.[10]

The present study concerns the effect of tolbutamide (Orinase†) and/or carbutamide (BZ-55‡) on glucagon hyperglycemia, on glucagonase and insulinase activity, and on the pancreatic morphology of the rabbit.

Materials and Methods

The experiments were divided into three groups.

(1) *Effect of tolbutamide on pancreatic morphology.* The study was carried out on 50 New Zealand white rabbits of both sexes whose weight varied from 2500 to 3000 gm. Of these animals, 6 served as controls; 4 received a single dose of tolbutamide (0.5 gm./kg.) and were sacrificed at 4 hr.; 12 animals received tolbutamide (0.5 gm./kg.) as a single daily feeding for 2 or 3 days; the remaining 18 animals were given 0.5 gm./kg. twice daily and were sacrificed at 1, 3, 4, 5, and 8 days after the start of the experiment, 4 hr. after the last dose of the drug.

The animals were maintained on Purina rabbit chow and water ad libitum. The tolbutamide was administered by intubation. The control animals were sham-fed. In 30 animals fasting specimens of blood were taken for glucose determinations in the morning prior to, and at 4 hours after, the administration of the drug. The rabbits were sacrificed by air embolization and the pancreas placed immediately into Zenker-formol (20 per cent) solution. Sections were stained by the Masson trichrome method, the chromalum hemo-

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† Kindly supplied by the Research Laboratory of The Upjohn Company, Ref. No. 307-REH-40B, 3107-PEM-100A, and 3305-ELP-33B.

‡ Kindly supplied by the Eli Lilly Research Laboratory, Ref. No. 1155-P-68057, 1155-68079, and 14989.

toxylin ponceau fuchsin method,[11] and the aldehyde fuchsin technique, counterstained with woodstain scarlet.[12]

(2) *Effect of sulfonylureas on the response to glucagon.* This study was conducted on both animals and humans. For the animal experiments, 16 New Zealand white rabbits weighing 2500 to 3000 gm. each were given a dose of 5 μg. of glucagon* intravenously after 16 hr. of fasting. The blood sugar response to glucagon given alone and administered 4 hr. after the intravenous administration of 0.25 to 0.5 gm./kg. of the sulfa derivatives was studied. Three fasting specimens of blood were taken for glucose determination at 10-min. intervals prior to the experiment. At that time either glucagon was given intravenously and the blood sugar response was noted, or else the sulfonylurea compounds were administered and glucagon was given 4 hr. later. In the latter experiment the blood sugar determinations were carried out hourly for 4 hr. after administration of the sulfa compounds and thereafter at 10, 20, 30, 45, 60, 90, and 120 min. after the glucagon injection. Whenever possible, these same animals were used for the control experiments and the two tests were performed about 1 week apart.

For the investigations in humans, we studied a group of 10 adults, 5 without diabetes and 5 with mild diabetes, who required no insulin. After a blood sample for the determination of fasting blood sugar had been withdrawn, each patient received 0.1 mg. or 0.5 mg. of glucagon intravenously, and the blood sugar was followed for the next 2 hr. Since repeated studies were carried out in several of these patients, a total of 15 tests was made. After an interval of 1 to 2 weeks these studies were repeated in 8 of these patients in exactly the same manner, except that either 2 or 3 gm. of tolbutamide or carbutamide was given orally about 2½ hr. prior to the administration of glucagon. The 2½-hr. interval was selected because, according to previous studies, the blood sugar is significantly lowered at that time.

The blood sugar determinations in the human and animal experiments were done by the Nelson modification of the Somogyi micromethod.[13]

(3) *Effect of sulfonylureas on glucagonase and insulinase activity*†. The *in vivo* studies were carried out after an 18-hr. fast on 17 New Zealand white rabbits weighing from 2500 to 3000 gm. each. Of these, 6 served as controls and 11 received the sulfonylureas either orally (0.25 to 0.5 gm./kg.) or intravenously (125 to 250 mg./kg.). I^{131}-labeled insulin or glucagon was administered 3 to 4 hr. following oral administration of tolbutamide or carbutamide and 1 to 1½ hr. following intravenous tolbutamide. The disappearance of the labeled hormone from the blood stream was followed by assay of radioactivity in washed trichloroacetic acid precipitates of the plasma.

The effect of sodium tolbutamide on insulinase, glucagonase, and adrenocorticotropinase activity was estimated using rat liver homogenates at concentrations of 0.017 mg./ml. and 0.30 mg./ml. Tolbutamide, at concentrations of 1 to 100 mg./ml., was added to mixtures containing I^{131}-labeled glucagon at a concentration of 0.2 μg./ml. Similarly, the effect of tolbutamide at concentrations of 0.1 to 2.5 mg./ml., on the rate of degradation of insulin-

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* Kindly supplied by Eli Lilly Research Laboratories, Lot No. 208-105B-197.

† This work was carried out in association with S. A. Berson and R. S. Yalow.[16]

I^{131} at concentrations of 0.5 to 100 mg. ml was estimated. Adrenocorticotropinase activity was also determined at various concentrations of tolbutamide.

Results

(1) *Morphologic studies.* As expected, the blood sugar levels declined after the administration of the sulfonylureas, and varied from 40 to 62 mg. per cent in a 4-hr period.

Morphologically, no changes were seen in either the α or D cells or in the exocrine portion of the pancreas. The β cells showed varying degrees of degranulation, which was intensified as the duration of therapy was prolonged. No other qualitative or quantitative alterations were found in these cells. After 1 day, very questionable reduction in granules was seen in an occasional animal. At 3 days, although some animals did not show any degranulation, a definite diminution of β cell granules was observed in several rabbits. At the fourth and fifth day this was further accentuated and, at 8 days, many of the animals showed very extensive or almost complete loss of their β cell granules (FIGURES 1 to 3). However, in no instance was β cell degranulation universal and, even at 8 days, an occasional animal showed only moderate or even no degranulation. As the β cells degranulated, a small rim of granules adjacent to the capillaries remained (FIGURE 2). The completely degranulated cell shows a thin aldehyde fuchsin positive cell border that allows its identification as a β cell.

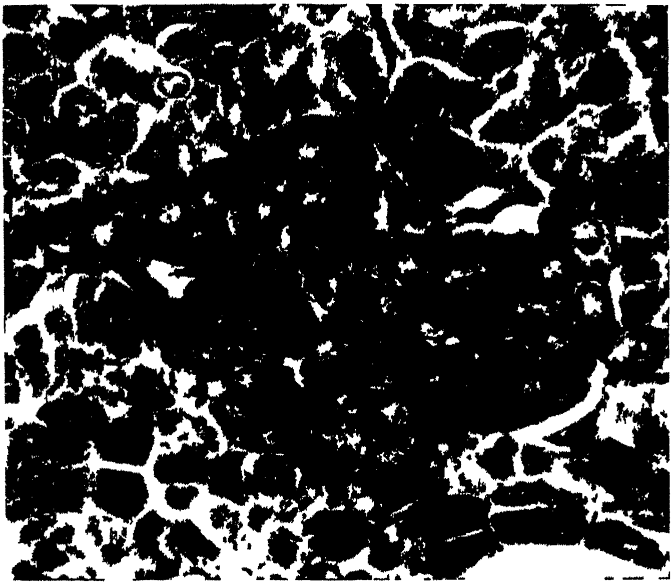

FIGURE 1. Islet of untreated rabbit, showing the normal arrangement of granules (black) along the capillary pole of the β cells. Aldehyde Fuchsin Woodstain Scarlet. (×520)

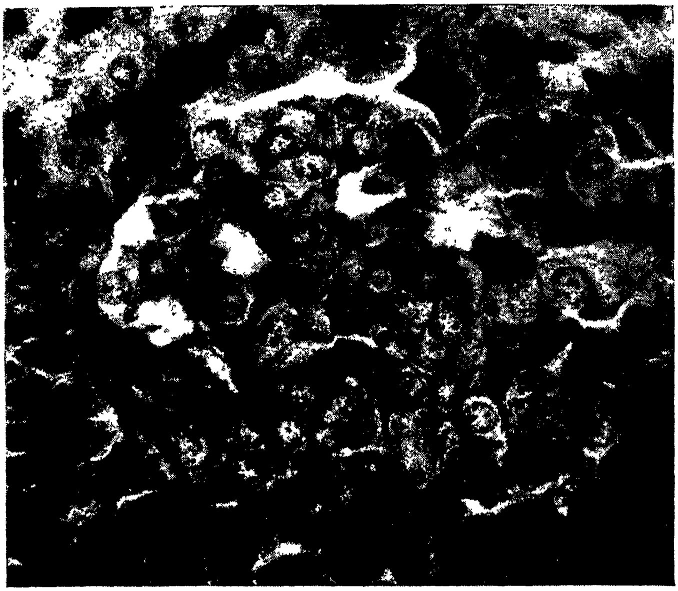

FIGURE 2. Islet of rabbit treated for 4 days with tolbutamide. There is definite degranulation, with residual granules collected at the capillary poles. Completely degranulated β cells show a thin aldehyde fuchsin positive (black) border. Aldehyde Fuchsin Woodstain Scarlet. (×520)

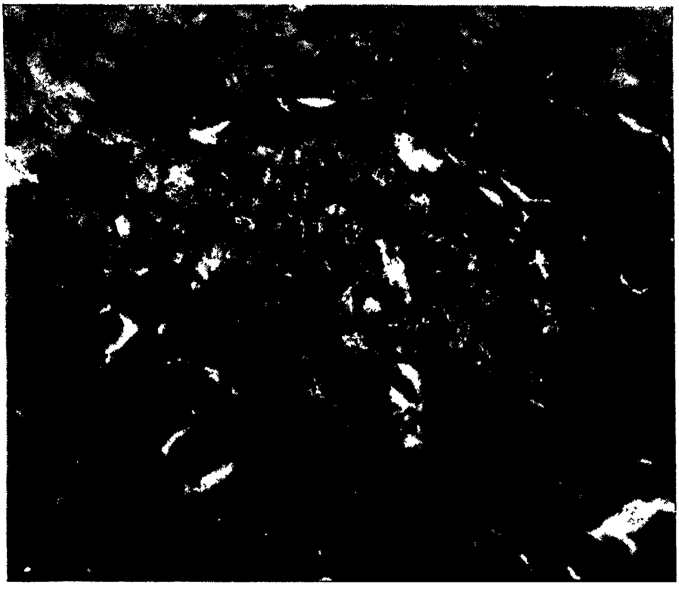

FIGURE 3. Islet of rabbit treated for 8 days with tolbutamide, showing complete degranulation. The β cells show a thin aldehyde fuchsin positive (black) border. Aldehyde Fuchsin Woodstain Scarlet. (×520)

(2) *Effect of sulfonylureas on the response to glucagon.* In tolbutamide-pretreated rabbits the maximal rise of the blood sugar after glucagon varied from +21 to +65 mg. per cent, with a mean of +46.1 (TABLE 1). After carbutamide administration, the mean maximum blood sugar elevation was 39.3 mg. per cent, with the range varying from +8 to +75 mg. per cent (TABLE 2). In the control rabbits the mean maximum hyperglycemia after glucagon was 58.5 mg. per cent; the maximum hyperglycemia was +140 mg. per cent. Statistical analysis disclosed no significant differences between the values obtained in the treated and in the control animals.

The hyperglycemic response to 0.1 mg. of glucagon was similar in the nondiabetic humans and in the well-controlled diabetic individuals (TABLE 3). The mean for the entire group was +53 mg. per cent, the range varying from +29 to +82 mg. per cent. The injection of each of the sulfa compounds 2½ hr. prior to the injection of glucagon caused no significant alteration of the blood sugar response, which in both groups of patients varied from +11 to +89 mg. per cent, with a mean of +44 mg. per cent (TABLE 4).

(3) *Effect of sulfonylureas on glucagonase and on insulinase activity.* The disappearance of precipitable radioactivity from the plasma of control and sulfonylurea-treated rabbits given insulin-I^{131} is demonstrated in FIGURE 4. In all but 2 of the sulfonylurea-treated animals the curves showed no distinct difference from those of the control rabbits. In 1 of the 2 exceptional cases the rabbit died during the experiment in spite of the administration of intravenous glucose 5 min. previously. In this case the slow disappearance of precipitable I^{131} is conceivably related to a lowered circulation rate due to shock. In the other exceptional case a control rabbit was unfortunately studied with a different lot of insulin-I^{131}.

TABLE 1

MAXIMUM BLOOD SUGAR RESPONSE AND TIME OF RETURN TO FASTING LEVEL OF 7 RABBITS AFTER INTRAVENOUS ADMINISTRATION OF 5 μG. GLUCAGON 4 HOURS AFTER INTRAVENOUS INJECTION OF TOLBUTAMIDE

Rabbit No.	Dose of tolbutamide (gm./kg.)	Maximum increase in blood sugar (mg.%)	Time of return to preinjection level (min.)
908	0.25	+43	>120
987	0.25	+65	72
959	0.25	+21	30
1085	0.25	+46	>120
1088	0.25	+54	50
1081	0.25	+53	59
1087	0.25	+41	>120
Total ...		323	571
Mean...		46.1	81.6
Normal control (mean) . .		58.5	85

TABLE 2

MAXIMUM BLOOD SUGAR RESPONSE AND TIME OF RETURN TO FASTING LEVEL OF 8 RABBITS AFTER INTRAVENOUS ADMINISTRATION OF 5 μG. GLUCAGON 4 HOURS AFTER INTRAVENOUS INJECTION OF CARBUTAMIDE

Rabbit No.	Dose of carbutamide (gm./kg.)	Maximum increase in blood sugar (mg. %)	Time of return to preinjection level (min.)
1005	0.5	+13	30
1004	0.5	+42	>120
1007	0.5	+75	>120
971	0.5	+62	>120
1017	0.5	+46	55
1013	0.5	+24	53
1019	0.5	+ 8	
1008	0.5	+44	>120
Total.............		314	618
Mean..................		39.3	88.3
Normal control (mean)..........		58.5	85

TABLE 3

MAXIMUM BLOOD SUGAR RESPONSE AND TIME OF RETURN TO FASTING LEVEL FOLLOWING INTRAVENOUS INJECTION OF 0.1 MG. GLUCAGON

Patient	Maximal increase in blood sugar (mg. %)	Time of return to pre-injection level (min.)
J. G.................	+61	51
S. K.................	+45	59
C. A.................	+60	83
S. G..	+59	
L. T.................	+59	>120
J. G.................	+43	90
M. F.................	+41	50
S. D.................	+53	>120
L. R. (S).............	+53	75
Y. G. (D)............	+29	84
Y. G. (D)............	+54	>120
M. G. (D)............	+66	>120
M. G. (D)............	+54	113
J. F. (D).............	+42	57
M. G. (D)............	+82	>120
Total................	801	1262
Mean..........	53.3	90.3

(D) = Diabetes
(S) = Steroid diabetes

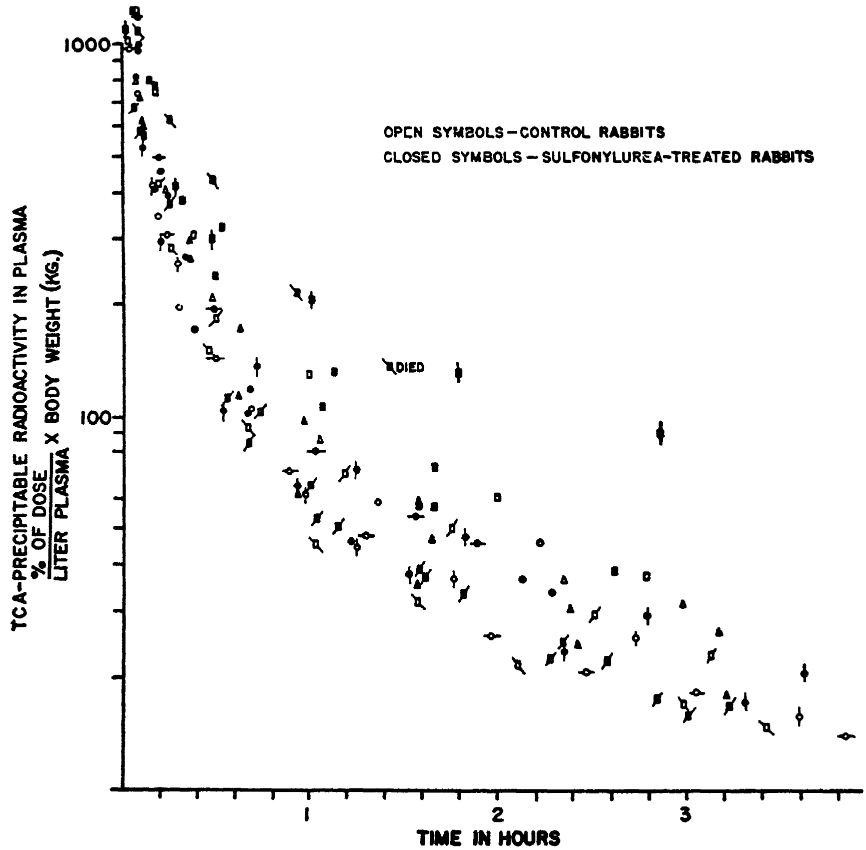

FIGURE 4. Disappearance of precipitable radioactivity from plasma following intravenous administration of insulin-I^{131} to control and sulfonylurea-treated rabbits. The top 2 "curves" were obtained from rabbits given tolbutamide. The remainder were given either tolbutamide or carbutamide. See text for details. Like symbols indicate same lot of insulin-I^{131}. (Reprinted with permission from *Diabetes*.[16])

The disappearance of glucagon-I^{131} was not significantly different in tolbutamide-treated and control rabbits. The blood sugar response to equal doses of glucagon likewise showed no definite differences, but in these studies the dose level of glucagon employed was above the threshold level.

The *in vitro* experiments did not reveal any detectable effect on liver insulinase or glucagonase activity at tolbutamide concentrations of 1 mg./ml. or less. At tolbutamide concentrations of 2.5 mg./ml., inhibition of liver insulinase appeared to be slight, but became progressively more marked at higher concentrations. At 100 mg./ml., inhibition of both insulinase and glucagonase activity was virtually complete. At this concentration adrenocorticotropinase activity was also completely inhibited.

TABLE 4
MAXIMUM BLOOD SUGAR RESPONSE AND TIME OF RETURN TO FASTING LEVEL FOLLOWING INTRAVENOUS INJECTION OF 0.1 MG. GLUCAGON GIVEN 2½ HOURS AFTER ORAL ADMINISTRATION OF ONE DOSE OF 2 TO 3 GM. TOLBUTAMIDE OR CARBUTAMIDE

Patient	Maximal increase in blood sugar (mg %)	Time of return to pre-injection level (min.)
S. K. (T)	+28	>120
M. F. (T)	+11	33
S. D. (T)	+82	>120
Y. G. (T) (D)	+34	45
M. G. (T) (D)	+89	>120
J. F. (T) (D)	+29	59
C. A. (C)	+56	88
M. F. (C)	+57	55
L. R. (C) (S)	+37	36
Y. G. (C) (D)	+17	28
Total	440	704
Mean	44 0	70.4
Control group (mean)	53.3	90.3

(T) = Tolbutamide
(C) = Carbutamide
(S) = Steroid diabetes

Discussion

The hypothesis that inhibition of glucagon action accounts for the hypoglycemic activity of the sulfonylurea preparations is not borne out by these or by previous findings.[14] In these experiments, neither in the rabbit nor in man was any significant difference observed in the degree or duration of glucagon-induced hyperglycemia with or without sulfa premedication. Moreover, it would seem that these drugs do not impair hepatic glycogenolysis, at least in response to glucagon. Furthermore, at moderate dosage levels we observed no effect of tolbutamide on the rate of degradation of I^{131}-labeled glucagon when administered intravenously *in vivo* or when studied with liver homogenates. The suggestion of Mirsky and his co-workers[6, 7, 15] that the mechanism for the blood sugar lowering effect of the sulfonylureas resides in an inhibition of the hepatic insulinase system is not borne out by the present findings. The experiments with I^{131}-labeled insulin showed that neither *in vivo* nor *in vitro*, except at high concentrations, is the rate of metabolic degradation of I^{131}-labeled insulin altered by tolbutamide or carbutamide.[16] Similarly, Vaughan,[8] working with an insulin concentration of 200 μg./ml. and with tolbutamide or carbutamide at concentrations of 0.13 to 0.18 mg./ml., failed to find any inhibition of insulinase activity in whole liver homogenates or in partially purified insulinase systems. In our experiments it was also seen that adrenocorticotropinase activity was inhibited when concentrations of tolbutamide of 100 mg. ml. were used. This may indicate that at high concentration the sulfonylureas may act as nonspecific enzyme inhibitors. Further evidence against the idea that carbutamide produces its hypoglycemic effect by inhibiting destruction of insulin has been reported by Fritz *et al.*,[17] who observed that acutely pancreatectomized dogs maintained with continuous infusions of insulin failed to respond to single intravenous injections of carbutamide.

The reported histological findings are at variance with the reports that the sulfonylurea derivatives act through destruction of the pancreatic α cells. This idea was advanced by the German workers,[4, 5] but has also more recently been negated by some of them.[18] These findings are also supported by observations that the sulfa compounds are effective in cortisone-treated[14] but not in severely alloxan diabetic animals,[5, 19] in both of which the α cells are intact. On the other hand, the lack of effectiveness in severe alloxan diabetes[5, 19] and in total pancreatectomy[2, 3] suggests an action via the β cells. This concept is, moreover, in conformity with the finding that the blood sugar is lowered in partially depancreatized and in some alloxan diabetic animals in which total destruction of the β cells has not been achieved,[20] and it is further borne out by the effectiveness of these drugs in hepatectomized animals.[21] These facts, taken together, tend to rule out a primary extrapancreatic action of the sulfonylureas, and suggest that the pancreatic β cells respond directly to sulfonylurea derivatives with an increased output of insulin. This idea[1, 2, 3] is further substantiated by the degranulation of the β cells.

However, this interpretation of the degranulation is made difficult by the fact that the degranulation is insignificant prior to the third day. Other workers[19] have taken this to mean that the degranulation that occurs after that time is induced in a way similar to that observed after prolonged insulin administration.[22] It has been considered a sign of functional rest of the β cells associated with an extrapancreatic hypoglycemic action of the sulfonylureas.[19] However, the previously reported experiments with chemically or surgically induced diabetes negate this explanation.

Although β cell degranulation correlates with pancreatic insulin content,[23] it does not reflect the rate of insulin formation or release.[24] Degranulation of these cells may occur under circumstances of diminished insulin production with normal storage, or of increased insulin release with normal or even increased production. The explanation mentioned above assumes diminution of insulin production as the cause of degranulation. Our interpretation, on the other hand, assumes increased insulin release with either normal, or possibly increased, insulin production. The evidence from studies in cortisone-treated rabbits supports the idea that increased insulin release may not be associated immediately with morphologically identifiable degranulation;[25, 26] in these studies 48 hr. or more were required before degranulation could be definitely identified. On the other hand, marked elevation of the blood sugar level by glucose administration may cause degranulation within 15 min.[27] or 3 hr.[28] To confirm the impression that the sulfonylureas increase insulin output by the pancreas, it will be important to measure their effect on the insulin content of the pancreatic veins. This has been attempted indirectly in experiments in which systemically ineffective doses of the sulfonylureas, perfused directly through the pancreas, caused significant hypoglycemia.[29]

The hypothesis that the sulfonylureas increase pancreatic insulin output, and the effectiveness of sulfonylureas in some diabetic patients, lead to interesting clinical implications. On this basis it may be assumed that these diabetic patients have sufficient capacity for insulin production and that the sulfonylureas act as more effective stimuli for insulin production or release than does hyperglycemia, which is not adequate in these instances.

These findings do not completely exclude additional extrapancreatic actions of the sulfonylureas, but these seem to be of secondary importance.

Summary

This study deals with certain possible mechanisms for the hypoglycemic action of carbutamide and tolbutamide.

Experiments were carried out to investigate the effect of premedication with sulfonylureas on the hyperglycemic response to glucagon. The results indicate that the hyperglycemic effect of glucagon is not inhibited by these drugs and that they do not influence hepatic glycogenolysis, at least in response to glucagon.

The administration of carbutamide (orally) and tolbutamide (orally and intravenously) to rabbits in dosage sufficient to induce significant hypoglycemia does not alter the rate of metabolic degradation of I^{131}-labeled insulin or glucagon. These findings indicate that the sulfa compounds do not act through inhibition of insulinase or stimulation of glucagonase activity.

No histological changes were observed in the exocrine portion of the pancreas nor in the α or D cells after administration of tolbutamide. Very mild and sporadic degranulation of the β cells was noticed during the first 3 days of treatment. After the fourth day, well-marked degranulation was present in some, and on the eighth day almost complete loss of the β cell granules was seen in many animals.

These observations suggest that the probable mechanism for the hypoglycemic action of the sulfonylurea compounds is stimulation of the pancreatic β cells to increase insulin output, which is reflected morphologically by their degranulation. However, these findings do not completely exclude additional extrapancreatic actions of the sulfonylureas, although these seem to be of secondary importance.

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TOXICOLOGICAL AND HISTOLOGICAL STUDIES WITH TOLBUTAMIDE

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In order to study the toxicological effects of tolbutamide* we fed rats and dogs high doses of this substance daily for a period of 9 months. Rats were given 250, 500, 1000, and 2000 mg./kg., respectively, by tube-feeding. A dosage of 2000 mg./kg. is equivalent to about 120 gm. daily administered to humans. Dogs received 100 mg./kg. daily over a period of 9 months; this dosage is high for dogs. When dogs are given 200 mg./kg. 5 to 7 times daily, they fall into hypoglycemic coma.

In rats, when doses up to 500 mg./kg. were given, there was no histological change in the internal organs nor in any glands of internal secretion except the pancreas. Only when the dosage exceeded 1000 mg./kg. was a fatty liver observed, and this did not show degeneration. The extent of the change in the liver paralleled the decrease of liver glycogen, as we have shown in earlier publications. Therapeutic doses, however, considerably increased the liver glycogen. In dogs, too, we observed no degenerative histological changes in the internal organs after an observation period of 9 months. Treatment with tolbutamide failed to alter the blood picture, nitrogen metabolism, or urine findings.

Recently, the degranulation of the β cells after the administration of tolbutamide has been the subject of much discussion. There are 3 possibilities: (1) Does tolbutamide cause a release of insulin from the β cells? (2) Does it cause a stimulation of the β cells, followed by exhaustion? (3) Does the degranulation indicate inactivity of the cells? Clarification of these questions is of considerable interest, particularly for the future of tolbutamide. If those authors who assume an exhaustion of the β cells are correct, our animals, which received extremely high doses for 9 months, should have become diabetic; that is, they should have shown a decreased glucose tolerance.

We discontinued administration of tolbutamide to the rats and dogs that had received it for 9 months and, 3 days later, we carried out glucose tolerance tests. The rats received 5 gm. of glucose per kg. intraperitoneally. After 1 hr., the control group of rats showed an increase in blood sugar of 210 per cent, while the tolbutamide-treated rats showed an increase of 180 per cent. After 5 hr., the blood sugars in both groups of rats had dropped to normal. Thus we see that the glucose tolerance is not decreased, but improved.

The same result was found in dogs. This is illustrated by the histological pictures. When daily doses of 2000 mg. of tolbutamide per kg. were given to rats over a period of 9 months some degranulation occurred. However, 12 days after the end of the administration of tolbutamide, the histological picture of the islet was again completely normal.

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* Tolbutamide (*N*-*p*-methylphenylsulfonyl-*N'*-butylurea), synthesized first in the laboratories of the Farbwerke Hoechst A.G., Frankfurt, is known under the following trade-marks: Rastinon-Hoechst, registered trade-mark of Farbwerke Hoechst A.G.; Artosin, registered trade-mark of Firma C. F. Boehringer u. Söhne GmbH, Mannheim; Orinase, registered trade-mark of The Upjohn Company, Kalamazoo, Mich. The code number applied by Farbwerke Hoechst A.G. for this substance during the experimental stage was D 860.

In a further series of tests we examined the degranulation after the administration of different doses to rats. When we gave 50 mg. per kg. and killed the rats 30 min. later, the histological sections showed slight degranulation. Sections taken 2 hr. after administration of the drug showed definite degranulation. The histological picture at 6 hr. was similar. At 24 hr. the granulation was more pronounced, and after 48 hr. the picture had returned to normal.

In an experiment with an extremely high dose (2000 mg. per kg.), we killed one group of rats after 6 hr. and found disappearance of the granulation. Animals killed after 24 hr. showed total degranulation. After 48 hr. the granulation was beginning to return to normal. After 72 hr. there was reappearance of the granulation and, after 96 hr., the normal picture appeared again.

We compared the degranulation after administration of tolbutamide with the degranulation after administration of glucose. Rats received 5 gm. kg. glucose orally. After 30 min., degranulation of the β cells was seen. After 2 hr. the granulation began to reappear. After 6 hr. the granulation was normal again.

In another series of experiments we fed rats 100 mg. of tolbutamide per kg. for 12 weeks, and killed the animals after 2, 4, 6, 10, and 12 weeks. The pancreatic islets of the rats killed after 2 and after 12 weeks showed normal granulation.

In the light of these results and, in part, those of the glucose tolerance tests, we conclude that tolbutamide neither destroys the β cells nor brings about a state of exhaustion of these cells.

SEX DIFFERENCES IN THE AMOUNT OF INSULIN EXTRACTABLE FROM DIABETIC HUMAN PANCREAS*

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Introduction

Inspection of the data now available indicates what appear to be sex differences in the amount of insulin extractable at autopsy from the diabetic human pancreas. In this paper these differences are identified, and their significance is evaluated.

Materials and Methods

These observations on the insulin extractable from the pancreas are based on totals of 90 male and 109 female diabetic human subjects studied individually. Included in these totals are 64 subjects reported on previously but not resolved by sex,[1] and also 6 male and 5 female diabetic subjects, studied by Scott and Fisher,[2] and for whom age at diagnosis and duration of diabetes were known. These studies were considered suitable for inclusion, since the same insulin extraction procedure[3] was used in both studies, and since it had been shown previously in the two series that no significant difference existed between the average amounts of insulin extractable from the pancreases of nondiabetic subjects following sudden death.[1]

All values for the amount of insulin extractable from the pancreas are expressed as units of insulin per kilogram of body weight at autopsy. Comparisons with levels of extractable insulin in nondiabetic subjects are made, using average values for unselected groups of nondiabetic male and female subjects whose pancreases were extracted and assayed in the same survey.[1]

Results and Observations

The amounts of insulin extractable from the pancreas in the individual male and female diabetic human subjects of the series are plotted vertically, as functions of age at diagnosis of diabetes and of its known duration, in FIGURES 1 and 2, respectively. An equation for the least squares plane of best fit† correlating years of age at diagnosis of diabetes, A, and the years of duration of life thereafter, D, with the amount of insulin extractable from the pancreas at autopsy, expressed as units per kilogram of body weight, I, has been calculated for each sex. For those persons diagnosed diabetic on or after their twentieth birthday (designated as maturity-onset diabetic subjects), the equations of these planes are:

$$I = (1.200) + (0.00437)A - (0.0460)D \qquad (1)$$

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† Multiple linear correlations.

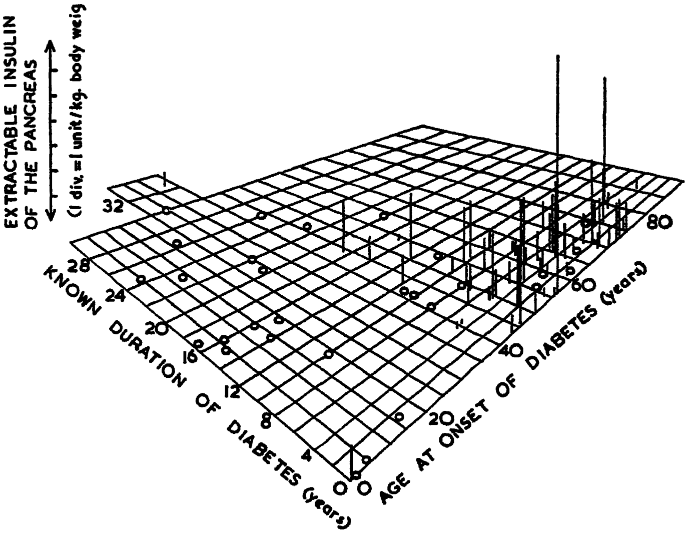

FIGURE 1. Extractable insulin of the pancreas at autopsy shown in relation to age at diagnosis of diabetes and to survival time thereafter. Male subjects.

TABLE 1

AVERAGE VALUES ± STANDARD ERROR FOR DURATION OF DIABETES, AND FOR AMOUNT OF INSULIN EXTRACTABLE FROM THE PANCREAS AT AUTOPSY FOR ALL GROWTH-ONSET AND MATURITY-ONSET DIABETIC HUMAN SUBJECTS OF THE SERIES. SUBDIVISIONS OF DATA ARE MADE BY SEX AND BY AGE AT DIAGNOSIS OF DIABETES

Age at diagnosis of diabetes	No. of subjects by sex	Duration in years		Level at which difference significant	Extractable insulin (units kg.)		Level at which difference significant
		Male Av. ± S.E.	Female Av. ± S.E.		Male Av. ± S.E.	Female Av. ± S.E.	
Growth-onset subjects 0–19.9 years .	13 M., 10 F.	10.5 ± 2.5	9.0 ± 2.3	*	0.16 ± 0.11	0.06 ± 0.03	*
Maturity-onset subjects 20.0–39.9 years.......	7 M., 9 F.	22.6 ± 3.6	16.1 ± 3.1	20%	0.15 ± 0.07	1.95 ± 0.68	5%
40.0–49.9 years.......	17 M., 22 F.	9.9 ± 1.5	13.7 ± 1.8	20%	0.94 ± 0.27	1.27 ± 0.21	*
50.0–59.9 years......	20 M., 27 F.	6.8 ± 1.3	8.7 ± 1.3	*	1.47 ± 0.48	0.87 ± 0.16	*
60.0–69.9 years.......	19 M., 24 F.	4.7 ± 0.8	5.2 ± 0.8	*	0.91 ± 0.17	0.99 ± 0.18	*
70 years and over.....	14 M., 17 F.	2.9 ± 0.7	4.2 ± 0.8	20%	1.49 ± 0.35	1.39 ± 0.26	*
All maturity-onset subjects................	77 M., 99 F.	7.7 ± 0.9	8.9 ± 0.8	*	1.10 ± 0.17	1.17 ± 0.11	*

* An asterisk indicates that the difference is not statistically significant.

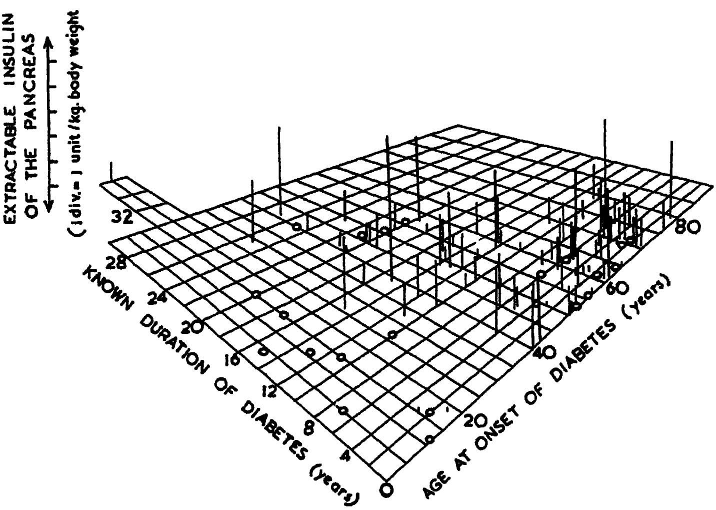

Figure 2. Extractable insulin of the pancreas at autopsy shown in relation to age at diagnosis of diabetes and to survival time thereafter. Female subjects.

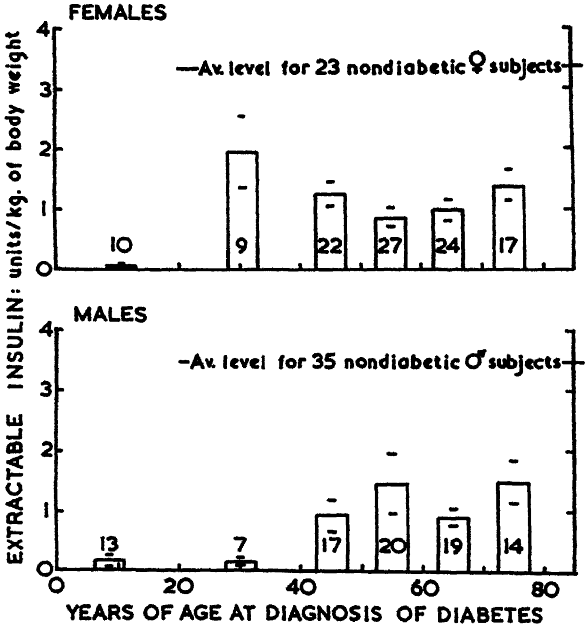

Figure 3. Average amounts of insulin extractable from the diabetic human pancreas at autopsy. Number of subjects and the standard error of estimate are shown for each group.

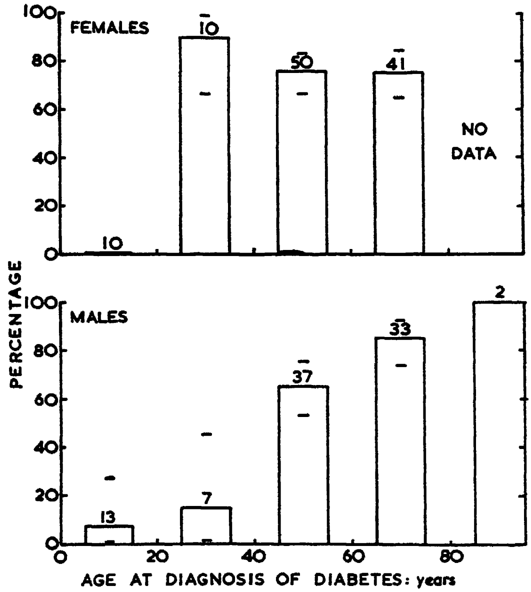

FIGURE 4. Variation with age at diagnosis of diabetes of the percentage of maturity-onset diabetic subjects with one tenth or more of the average amount of insulin found at autopsy in adult nondiabetic subjects of the same sex. Number of subjects and 10 per cent confidence limits are shown for each group.

for the 77 male subjects;

$$I = (1.198) - (0.00044)A + (0.00025)D \tag{2}$$

for the 99 female subjects.

In TABLE 1 all diabetic subjects of the series are grouped by age at diagnosis of the diabetes for each sex. The statistical significance of sex differences in the group-average values for known duration of diabetes and amount of insulin extractable from the pancreas is shown. These comparisons are illustrated in FIGURE 3.

It has been noted previously[1] that grouped values for the amount of insulin extractable from *diabetic* human pancreases do not have a normal distribution, an assumption that is involved in making the above calculations. For this reason an alternative basis for analysis has been set up. The diabetic human subjects within a given class interval were subdivided into those having as much as or more than, and those having less than, a specified small fraction (5, 10, 20 per cent) of the average amount of insulin extractable from the nondiabetic human pancreas*. The percentage of grouped subjects having as much as or more than the specified fraction was determined, and the confidence limits of this percentage were obtained from statistical tables compiled by Mainland.[8]

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* These reference levels were selected because diabetes becomes manifest only after the surgical removal of all but a small portion (5 to 20 per cent) of the pancreas from mice,[4] rats,[5] and dogs,[6] and only when the extractable insulin of the pancreas in rats has been reduced into (or below) this range by alloxan.[7]

The results of this type of analysis, in which one tenth of the nondiabetic level of extractable insulin was selected as the dividing line are shown in FIGURES 4 and 5. The corresponding patterns obtained when the dividing level was set at one twentieth or at one fifth, and the conclusions deducible from them, corresponded reasonably well with those shown in FIGURES 4 and 5, and are not shown.

An analysis similar in nature to those just described was performed using Hartroft's[9] counts of the number of islets of Langerhans per unit area in histological sections with a thickness of three μ. This partial analysis included 66 of the maturity-onset diabetic subjects of the present series. The findings are illustrated in FIGURE 6. A similar analysis was made using the percentage falls in blood sugar level for diabetic subjects following a single standard dose of tolbutamide, observed by Mirsky, Diengott, and Dolger.[10] These are illustrated in FIGURES 7 and 8.

Regardless of whether the analyses of data are based on values for the absolute amount of insulin extractable from the pancreas or upon percentages of subjects with very low levels of this factor, statistically significant differences between the sexes can be observed. The linear regression shown in

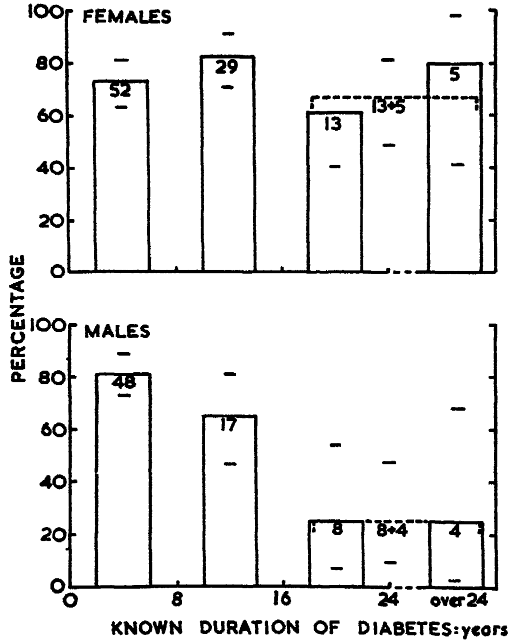

FIGURE 5. Variation with known duration of diabetes of the percentage of maturity-onset diabetic subjects with one tenth or more of the average amount of insulin found at autopsy in adult nondiabetic subjects of the same sex. Number of subjects and 10 per cent confidence limits are shown for each group.

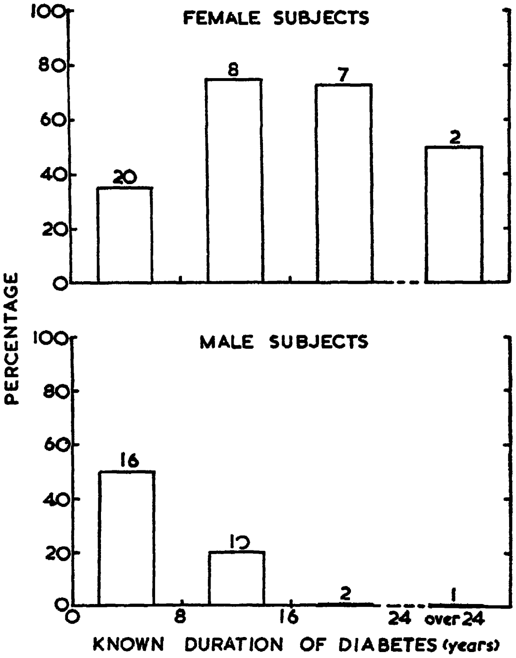

FIGURE 6. Percentages of diabetic human subjects with more than 3.5 islets of Langerhans per sq. mm. of tissue section. Data of Hartroft and Wrenshall.[9] Number of subjects is shown at the top of each column.

EQUATIONS 1 and 2 between extractable insulin, I, and age at diagnosis, A, is significant at the 10 per cent level for male but not for female subjects. The degree of linear regression between I and duration of the diabetes is significant at the 5 per cent level in males and does not differ significantly from zero for females. The average level of the extractable insulin of the pancreas in female subjects diagnosed diabetic in the 20.0 to 39.9 year age range is significantly higher than that for the corresponding male subjects (TABLE 1). For male subjects diagnosed diabetic in the 20.0 to 39.9 year age range, the percentage of subjects with one tenth or more of the extractable insulin of pancreas found in the nondiabetic control group is significantly lower than that for diabetic female subjects in the same age range at diagnosis (FIGURE 4). Similarly, the percentage of male subjects who have survived for more than 16 years after having been diagnosed diabetic differs at the 10 per cent level of significance from that for female subjects of the same description (FIGURE 5).

Discussion

The amount of insulin extractable from the pancreas[11–14] and the volume of islet tissue[15] have been reported to increase in experimental animals as a result of the administration of certain estrogens but not after administration of the androgen testosterone. The action of the ovaries and of estrogens,

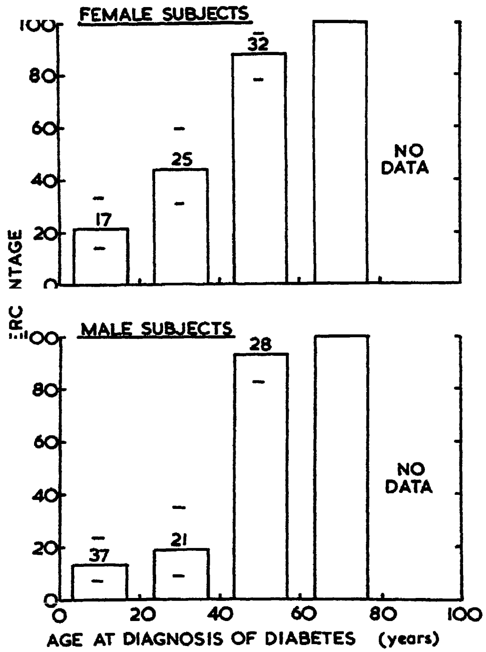

FIGURE 7. Percentages of diabetic human subjects for whom the fall in blood sugar level was more than 20 per cent in the single-dose tolbutamide test of Mirsky, Diengott, and Dolger.[10] Number of subjects is shown at the top of each column.

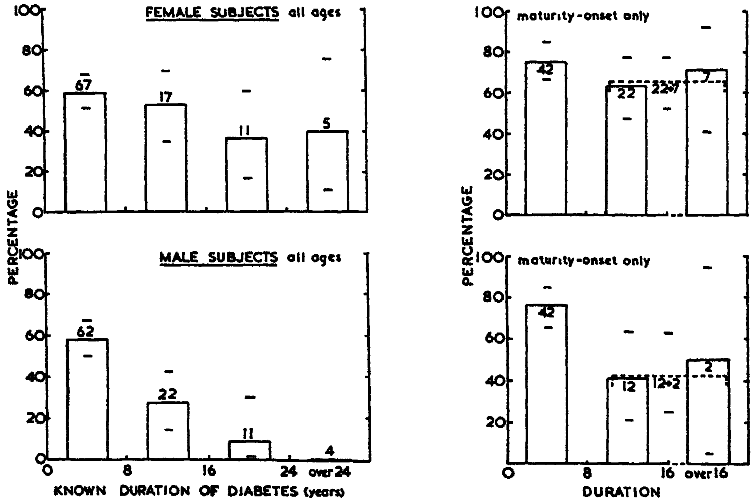

FIGURE 8. Duration of diabetes versus percentage of diabetic human subjects in whom the fall in blood sugar level was 20 per cent or more in the single-dose test of Mirsky, Diengott, and Dolger.[10] Number of subjects and 10 per cent confidence limits are shown for each group.

especially when associated with insulin therapy in preventing the development of diabetes in the 95 per cent depancreatized rat or in overcoming alloxan diabetes, is accompanied by hyperplasia and an increase in the number of islets of Langerhans.[16]

In comparison with this, it is of interest to note that the highest average level of extractable insulin of the pancreas in diabetic female subjects occurs in the 20.0 to 39.9 year age group at diagnosis (TABLE 1) and that this level is higher (but only at the 20 per cent level of significance) than that for female subjects 50.0 to 59.9 years of age at diagnosis of diabetes. The 20.0 to 39.9 year age range is also the interval of greatest fertility in women, while this phase of life has been passed in women over 50 years of age.

The changes in the pancreas remnant produced by sex factors take appreciable time to develop.[16] The same appears to be true for diabetic man. According to EQUATION 1 the average rate of reduction in the extractable insulin of the pancreas in maturity-onset diabetic man amounts to about 4 per cent per year of the average level in the newly diagnosed subject. This is in contrast to our experience of almost complete loss of extractable insulin (and of β cells) within one year in male and female human subjects diagnosed diabetic while growth is actively continuing,[17] presumably under the action of pituitary somatotropin. It is recognized that the basis for the above comparisons between animals susceptible to diabetes and diabetic human subjects leaves much to be desired.

The close similarity in the patterns of FIGURES 5 and 6 is what would be expected if the fall to very low levels in the extractable insulin of the pancreas were caused by loss of islet tissue. This possibility is currently under investigation, using histological sections of pancreas from all subjects of the series.

With the exception of the paper by Mirsky, Diengott, and Dolger,[10] detailed comparisons of the responses of male and female diabetic human subjects to sulfonylurea therapy have not been found in the literature. Using an analysis of covariance in their series of 100 male and 100 female diabetic subjects, these authors found no statistically significant differences between the means or between the regression coefficients of six independent factors, including age at diagnosis and duration of diabetes in the two sexes.

In view of the lack of correspondence in this and other details in the patterns of response of the diabetic subjects of their series to a single test dose of tolbutamide and of the amounts of insulin extractable from the pancreas at autopsy in the subjects of our series, a comparison of original data was arranged. Enumeration analyses of the tolbutamide test data, similar in types to those illustrated in FIGURES 4 and 5, are shown in FIGURES 7 and 8, respectively.

A noteworthy difference between the two sets of data is a relative lack of subjects of either sex with maturity-onset diabetes of long duration in the tolbutamide series. In our series there are 10 male and 15 female subjects in this category who survived 18 years or longer following diagnosis of diabetes while, in the series of Mirsky, Diengott, and Dolger, only 1 male and 6 female subjects fall into this category. Since duration of diabetes in male subjects proved to be the major factor correlating with the extractable insulin levels in our series, the lack of such long-term subjects in the tolbutamide series may account for the apparent lack of a significant degree of correlation in this regard. However, even with this limitation, there are indications that the proportion of maturity-onset male subjects responding favorably to the tolbutamide test is significantly less after the diabetes has lasted 8 or more years than in the corresponding female group (FIGURE 8). Additional tolbutamide-response tests performed on male and female subjects diagnosed diabetic in the 20 to 40 year age range, and with diabetes of long duration should serve to clarify this situation.

Summary and Conclusions

The amount of insulin extractable from the pancreas at autopsy has been measured individually in 90 male and 109 female diabetic subjects, using standardized procedures. Sex-specific analyses of the covariance of age at diagnosis, A, and duration of diabetes, D, with extractable insulin, I, have been performed. Significant degrees of linear correlation between I and D and between I and A have been established for male (but not for female) subjects diagnosed diabetic after the first two decades of life. All but recently diagnosed subjects of both sexes diagnosed diabetic in the first two decades of life had very low levels of extractable insulin.

The proportion of subjects with low levels of extractable insulin (under 10 per cent of the nondiabetic average) has been determined for each sex in class intervals relating to A and D. For diabetes diagnosed after the second decade of life, the proportion of female subjects with low levels of pancreatic insulin was small, and did not vary greatly with either A or D. In contrast, the diabetic male subjects showed a progressive decrease with A and a progressive increase with D in the proportion of subjects with low levels of extractable insulin.

These findings have been compared with sex differences seen in the pancreas in experimental diabetes, and in the clinical responses of diabetic subjects to sulfonylurea therapy, published by others.

Acknowledgment

We are grateful to R. E. Haist for a number of valuable suggestions concerning the presentation of data and to I. A. Mirsky and his colleagues for permission to refer to their original data. Mrs. Beverly Schaeffer and Mrs. Christina Goodridge took part in the collection and processing of the data.

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REPORTS ON STUDIES WITH CARBUTAMIDE AND TOLBUTAMIDE DONE AT THE CHARLES H. BEST INSTITUTE, UNIVERSITY OF TORONTO

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The combined report that follows consists of summaries of work recently done with carbutamide and tolbutamide in five different sections of the Charles H. Best Institute. I have been delegated to summarize and present the findings of the research teams involved. The names of the contributors are indicated with each summary.

D. W. Clarke has measured glycogen levels in rat diaphragms before and during incubation *in vitro* following the *in vivo* administration of carbutamide or tolbutamide. Six groups of normal rats received daily injections of either saline, 50 mg. carbutamide, 50 mg. tolbutamide, 10 mg. cortisone, carbutamide and cortisone, or tolbutamide and cortisone. After 14 days, the rats were killed and the diaphragms were removed for a determination of their initial glycogen. The amounts of glycogen synthesized after incubations in a glucose-containing medium were also determined.

With 7 animals per group, the values for the initial glycogen levels were as shown in TABLE 1. Treatment with carbutamide significantly increases the initial glycogen. The apparent increase with tolbutamide is not statistically significant, although it must be considered suggestive. The effect of cortisone treatment was to increase initial muscle glycogen. There were no interactions between cortisone and either tolbutamide or carbutamide.

Average values for net glycogen synthesis are shown in TABLE 2. In many cases there was actually less glycogen after than before incubation, and these cases are shown in TABLE 2 as *negative* values for net glycogen synthesis. The greater glycogen loss in the carbutamide-treated animals than in the saline-injected controls is statistically significant. The results with the tolbutamide-treated animals are not significantly different from those obtained with control animals.

It was demonstrated previously that there is no direct effect of carbutamide in stimulating glycogen synthesis of the diaphragm.[1] The effect noted

TABLE 1

INITIAL GLYCOGEN CONTENT OF RAT DIAPHRAGM IN MG./GM. OF MUSCLE FOLLOWING PRETREATMENT WITH SALINE, CARBUTAMIDE, OR TOLBUTAMIDE, WITH AND WITHOUT CORTISONE

	Saline	Carbutamide	Tolbutamide
No cortisone	0.97	1.29	1.28
With cortisone	2.19	2.68	2.09

Table 2

Net Glycogen Synthesis in Rat Diaphragm in mg. gm. of Muscle Following Pretreatment with Saline, Carbutamide, or Tolbutamide, with and without Cortisone

	Saline	Carbutamide	Tolbutamide
No cortisone........ . . .	0.16	−0.16	−0.07
With cortisone ...	−0.82	−1.41	−0.83

here, obtained by repeated daily injections with carbutamide, may possibly result from increased peripheral insulin activity.

The effects of tolbutamide and carbutamide on the acetylation of *p*-nitroaniline by a soluble pigeon liver enzyme system in the presence of coenzyme A (CoA) has been studied by H. Socol, E. Schönbaum, and J. Campbell. These experiments were incidental to a long-range study on the role of CoA in metabolism. The method used was a slight modification of the procedure

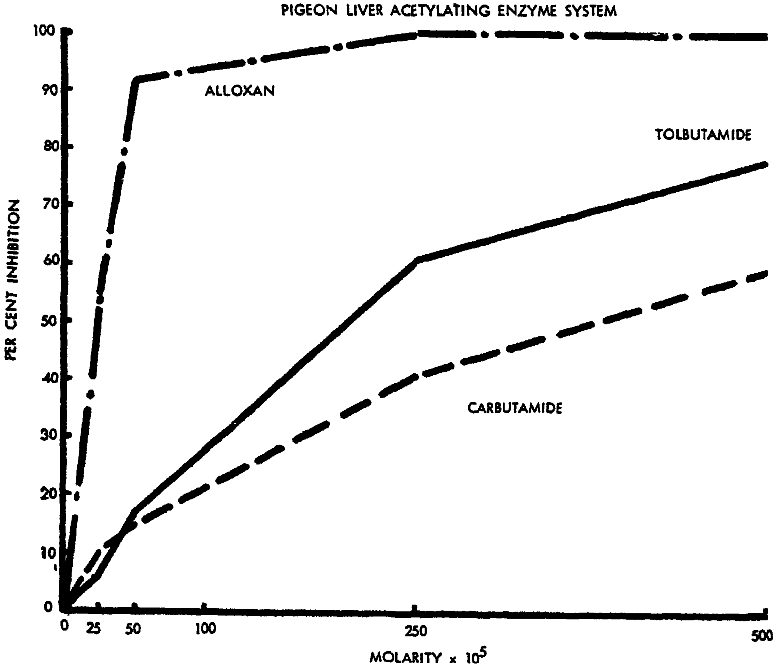

Figure 1. Acetylation of *p*-nitroaniline. Each value represents the average of at least two determinations. Contents of each tube included 19 μmoles of sodium citrate, 24 μmoles of sodium acetate, 4 μmoles of adenosine triphosphate, 0.4 μmole of *p*-nitroaniline, 100 μmoles of tris(hydroxymethyl)aminomethane buffer at *p*H 7.6, 15 μmoles of cysteine hydrochloride, 1.5 Lipmann units of coenzyme A, 0.2 ml. of Dowex-treated enzyme solution (10 per cent), 0.2 ml. of inhibitor and/or water, in a total volume of 1.1 ml.; incubated for 1 hr. at 37° C.

of Kaplan and Lipmann,[2] using *p*-nitroaniline (4×10^{-4} *M*) as acceptor instead of sulfanilamide. Either tolbutamide or carbutamide was added in multiples of 2.5×10^{-5} *M*. Urea and some of its derivatives of biological interest were also included in this series. Barbiturates, in concentrations of 10^{-3} *M*, have been reported recently to inhibit acetylation.[3]

As seen in FIGURE 1, both tolbutamide and carbutamide inhibit acetylation strongly at concentrations around 2.5×10^{-3} *M* (approximately 72 mg. per cent). At a concentration in the enzyme system lower than may be produced in blood during treatment, the inhibition is already noticeable. Carbutamide itself is partly acetylated by this enzyme system. At a final concentration of 2.5×10^{-3} *M*, 4.5 per cent of the total carbutamide is acetylated, and at double this concentration 16 per cent is acetylated. Therefore, competition for acetyl groups appears to occur at the higher concentrations of carbutamide. The acetylating enzyme system was strongly inhibited by alloxan, near-maximal inhibition occurring even at a concentration of 5×10^{-4} *M*.

Of other substances tested, barbiturates (pentobarbital, amobarbital sodium) had relatively small inhibitory effects, and diphtheria toxin, synthalin, guanidine, and urea were without effect (TABLE 3). The toxin was included because it is known to produce adrenal lesions.

The basic modes of action of carbutamide are not yet clear. Several pieces of evidence gathered by M. A. Ashworth, Rosemary D. Hawkins, and R. E. Haist point to a stimulation of the islets of Langerhans as one of its

TABLE 3
INHIBITION OF *p*-NITROANILINE ACETYLATION

Inhibitor	Molarity	Per cent inhibition
Tolbutamide	2.5×10^{-5}	0
	5.0×10^{-5}	4
	2.5×10^{-4}	6
	5.0×10^{-4}	17
	2.5×10^{-3}	61
	5.0×10^{-3}	78
Carbutamide	2.5×10^{-5}	7
	5.0×10^{-5}	4
	2.5×10^{-4}	10
	5.0×10^{-4}	15
	2.5×10^{-3}	41
	5.0×10^{-3}	59
Alloxan	2.5×10^{-4}	54
	5.0×10^{-4}	92
	2.5×10^{-3}	100
Pentobarbital	2.5×10^{-4}	0
	2.5×10^{-3}	28
Amobarbital sodium	2.5×10^{-4}	0
	2.5×10^{-3}	23

Synthalin, guanidine, urea, and diphtheria toxin: no inhibition.

actions. Ashworth and Haist[4] showed that when rats were given carbutamide by mouth daily for from 3 to 5 wk. at doses of 0.5 to 1.0 gm./kg. of body weight, an increase in islet weight was noted. Previously, B. Kinash[5] found that continuous intravenous infusion of carbutamide at the rate of approximately 1 gm. kg. day for one week did not lead to a significant increase in islet tissue. With the longer period of oral administration of the carbutamide, however, there was an increase in the islet weight. This was significant at the 1 per cent level. At the same time there was a significant decrease in pancreatic weight and thus an increased concentration of islet tissue in the pancreas. The islet weight per unit of body weight was also significantly increased.

This finding supports the view that carbutamide somehow, directly or indirectly, stimulates the islets of Langerhans and that this stimulation persists over a period of time.[6] Evidence which is harmonious with the foregoing was obtained from studies on glucose-6-phosphatase. Hawkins, Ashworth, and Haist[7] found that administration of carbutamide to rats for 3 weeks led to a reduction in the glucose-6-phosphatase activity of the liver. Since it has been reported that insulin reduces the glucose-6-phosphatase activity of the liver,[8] there was a possibility that this effect of carbutamide was due to insulin release. For this reason Hawkins has investigated the effect of carbutamide on the glucose-6-phosphatase activity of the liver in rats made diabetic with alloxan. In these diabetic rats no reduction in the glucose-6-phosphatase activity of the liver was observed following 8 days of administration of carbutamide; indeed, a rise was evident. With the same period of administration of carbutamide the normal, nondiabetic rats showed good reductions in glucose-6-phosphatase activity. The rise in glucose-6-phosphatase activity after alloxan, previously reported by other groups,[8] was observed also. The results of this experiment indicate that insulin is required for the effect of carbutamide on the glucose-6-phosphatase activity of liver, but whether or not an increased amount of insulin is necessary for this effect is not known at present. However, these results are in harmony with the view that carbutamide stimulates the secretion of insulin by the islets.

The actions of carbutamide and of tolbutamide on the function and histological picture of the thyroid gland of the rat have been studied by J. Logothetopoulos and J. M. Salter. In this study a search has been made for a possible relationship between the "hypoglycemic" and "thyroid" effects of these substances.

Carbutamide, tolbutamide, sulfadiazine, and sulfapyridine were tested for their inhibitory effect on uptake of I^{131} by the thyroid gland. Carbutamide (200 mg.) and equimolar doses of the other compounds were injected subcutaneously into groups of 8 rats 0.50 hr. before an intraperitoneal injection of 10 μc. of I^{131} as sodium iodide. The thyroid glands were removed 4.0 hr. later and the total radioactivity in their digests was counted. The I^{131} uptakes, expressed as percentage of the uptake in the control group, are shown in TABLE 4.

The marked difference in the effect of the two sulfonylureas on I^{131} uptake,

TABLE 4

COMPARATIVE INHIBITION OF I^{131} UPTAKE BY THE RAT THYROID GLAND CAUSED BY THE INJECTION OF VARIOUS AGENTS INCLUDING TOLBUTAMIDE AND CARBUTAMIDE

Group	Thyroid uptake of I^{131} (mean as per cent of controls ±S.D.)
Controls	100 ± 30
Carbutamide	10 ± 3
Tolubutamide	61 ± 13
Sulfadiazine	6 ± 2
Sulfapyridine	15 ± 6

seen in TABLE 4, was paralleled by the difference in their goitrogenic properties. The carbutamide or tolbutamide was given at 0.2 gm. per kg. body weight in two injections daily for 21 days to groups of 10 rats, kept on a low-iodine diet.

Tolbutamide did not induce any change in the weight or the histological picture of the thyroid gland, but the rats treated with carbutamide developed goitrous glands with all the histological signs of release of pituitary thyroid-stimulating hormone. The thyroid weights (±S.D.) in milligrams for the control and treated groups were: 40 ± 9 for the carbutamide group, 23.5 ± 4.1 for the tolbutamide group, and 23.5 ± 3.5 for the controls.

The goitrogenic effect of carbutamide was fully prevented by a daily injection of 4 μg. of L-thyroxine. The thyroxine-supplemented carbutamide-treated group showed the same blood sugar levels after the last injection of the sulfonylurea and the same pattern of loss of stainability in β-cell granules as did the goitrous group on carbutamide alone. These findings, together with the fact that tolbutamide, while lacking a "thyroid" effect, had as strong a hypoglycemic action as carbutamide, indicate: (1) that the two actions may be due to different parts of the molecule, and (2) that the pituitary-thyroid imbalance does not contribute significantly to the effect of the hypoglycemic sulfonamides on carbohydrate metabolism.

Severe hepatotoxic effects have been observed by Anna Sĭrek, O. V. Sĭrek, and C. H. Best in depancreatized dogs and puppies treated with carbutamide for extended periods. Earlier findings relating the administration of carbutamide to changes in blood lipid and the blood and urine sugar levels in adult animals have been reported elsewhere.[9] Since then it has been observed that this drug, when chronically administered in "maintenance doses," interfered seriously with the blood-clotting mechanism of these animals.

This observation has been extended to include two littermate depancreatized puppies. The blood prothrombin levels were decreased below pre-carbutamide control levels following 39 and 63 days on 0.5 to 1.0 gm. carbutamide per day for the depancreatized pups and dogs, respectively. Dog No. 1 and both puppies died under the condition described above on the sixty-fourth, fortieth and forty-second days. Post-mortem findings on these three animals, together with those on a depancreatized hypophysectomized dog, which showed bleeding tendencies but in which no prothrombin time had been measured, revealed subendocardial petechiae and subserosal hemorrhages throughout the body in two animals. There was no evidence of brain hemorrhages. A common finding in all four animals was that the liver was enlarged and fatty. In the puppies it was very large, yellow, and friable. The findings of bleeding tendencies and fatty livers in depancreatized adult dogs treated with carbutamide find support in similar observations made by P. Schambye[10] in the laboratory of K. Hallas-Møller in Denmark. These combined results are interpreted as being caused by a toxic action of carbutamide on liver tissues.

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LONG-TERM STUDIES OF THE SULFONYLUREAS IN TOTALLY DEPANCREATIZED DOGS*

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The hypoglycemic action of carbutamide (BZ-55) and tolbutamide (U-2043, Orinase) has not been satisfactorily explained. Their ineffectiveness in the absence of insulin proves that they are not substitutes for that hormone.

The completely pancreatectomized animal treated with suboptimal amounts of insulin is a useful preparation for exploring some of the routes by which the compounds may act. If these compounds lowered the blood sugar under these conditions, it would be demonstrated that neither stimulation of the β cells nor inhibition of the α cells of the pancreatic islets is an essential part of the mechanism. It might then be concluded that the injected insulin itself is potentiated or that the primary effect is on other tissues involved in carbohydrate metabolism, with insulin playing only a permissive role. On the other hand, if such experiments were negative, it would mean that the presence of the pancreas is necessary, and the possibilities that the drugs influence insulin secretion or α-cell function would remain open.

Observations of this kind have been few in number, variable in design, and inconsistent in outcome. Root[1] has reported a 50 per cent fall in blood sugar after oral administration of a single dose (2 gm.) of carbutamide to a totally depancreatized dog receiving 10 units of NPH insulin daily. Sîrek and Sîrek[2] studied 2 such animals for more than 2 months. Insulin was reduced at the same time that feeding of carbutamide was begun, and the results were judged by changes in "insulin requirement," a criterion that must be employed with caution. One dog seemed to show some effect of the drug, although the authors minimize its significance. The other dog, on daily treatment with carbutamide, eventually was able to achieve normal and even subnormal levels of blood sugar with as little as 1 unit of insulin given at intervals of up to 10 days. There is no reference to post-mortem verification of the completeness of pancreatectomy. Campbell,[3] after a suitable control period, administered carbutamide daily over a period of 43 days to a pancreatectomized dog maintained on constant diet and insulin dosage, and found a definite decrease in fasting blood sugar levels and subsidence of glycosuria to zero. Withdrawal of insulin with continuance of the drug resulted in hyperglycemia, glycosuria, and ketosis. On the other hand, Fritz and his colleagues,[4] in acute experiments on depancreatized dogs, were unable to demonstrate any enhancement of activity of intravenously infused insulin when a single 1 gm. dose of carbutamide was given by the same route. The experiments were performed under pentobarbital anesthesia.

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Methods

Six mongrel dogs weighing 8.9 to 12.7 kg. were pancreatectomized, with every attempt at completeness, under pentobarbital anesthesia. Following recovery, diet* and insulin were adjusted over a period of 2 to 7 weeks with the purpose of permitting well-marked hyperglycemia and glycosuria without undue loss of weight. Thereafter, with minor exceptions, conditions were kept constant. The experiments were planned so that a control period without drug of from 8 to 22 days was followed by an experimental period with drug of from 5 to 17 days, then by a recovery period, a second experimental period, and a final recovery period. Carbutamide was given in the first experimental period and tolbutamide in the second in doses of 1 gm. daily, except for 1 dog that received 3 gm. daily.

The animals were kept in metabolism cages. Food, regular insulin, and drugs (*per os*) were given twice daily in equal amounts. Daily 24-hr. collections of urine were analyzed quantitatively for glucose,[5] and fasting blood sugar levels[6] were determined as a rule 3 times per week.

Results

Three dogs (Nos. 5, 6, and 8) completed the experiments as originally planned. Dog 5 died, presumably of inanition, 5 days after the close of the final recovery period. Autopsy revealed extreme cachexia with atrophy of the thyroid and myocardium. A small nodule on the wall of the duodenum contained no pancreatic tissue in frozen sections, and none was found elsewhere despite careful searching.

The remaining 3 dogs died during the observations and merit the following comments:

Dog 2, the only animal to receive 3 gm. of carbutamide daily, died 5 days after the drug was discontinued, having ingested a total of 16 gm. during 5½ days. Before death the dog displayed anorexia, weakness, vomiting, jaundice, and orange-colored urine. At autopsy the body fat was stained orange. The liver was fatty. Degenerative changes were present in the renal tubules, and both these and Bowman's spaces contained considerable protein. No pancreatic remnants were found on gross or microscopic examination.

Dog 7 was found dead in its cage the day after it had completed an 11-day course of treatment with 1 gm. of carbutamide daily. Three days before death the animal became somewhat lethargic and its appetite was diminished. The urine was orange in color, but tests for bile, bilirubin, urobilinogen, por-

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* The daily diet, kept constant for each dog after stabilization, consisted of 1½ to 3 cans (net weight of 1 can = 480 gm.) of Rival Dog Food plus 20 to 30 gm. of pancreatin powder (Lilly). Ingredients of the canned food are said to be meat products, including liver, soybean flakes, soybean oil meal, meat meal, barley, wheat germ, wheat, iodized salt 0.5 per cent, sodium nitrite 0.0004 per cent, sodium nitrate 0.0003 per cent, iron oxide 0.05 per cent, onion and garlic powder, and water. "Guaranteed analysis" is said to show: *minimum*, crude protein 11.5 per cent, crude fat 2.5 per cent, nitrogen-free extract 7.5 per cent, Ca 0.3 per cent, P 0.3 per cent; *maximum*, crude fiber 1 per cent, crude ash 3 per cent, salt 0.5 per cent, moisture 74 per cent.

TABLE 1

Dog No.	Wt. (kg.) Orig. Term.	Daily insulin (units)	Control period		Experimental period			Recovery period		Experimental period			Recovery period		Remarks
			Av. bl. sug. (mg. %)	Av. ur. sug. (gm.)	Drug dose (gm.)	Av. bl. sug. (mg. %)	Av. ur. sug. (gm.)	Av. bl. sug. (mg. %)	Av. ur. sug. (gm.)	Drug dose (gm.)	Av. bl. sug. (mg. %)	Av. ur. sug. (gm.)	Av. bl. sug. (mg. %)	Av. ur. sug. (gm.)	
2	11 8 —	6 P	11† 290*	52	Carb. 3	5 320* 63*	4	5 241	5						Died after 16 gm. of drug in 5½ days. Anorexia, vom., jaundice, fatty liver, renal tub. degeneration. No gross or micro. panc. tissue.
5	11 0 5 9	0 4 R	14 262 22 294	47 29	Carb. 1 Carb. 1	17 251 14 249	44 11	8 278 14 279	47 22	Tol. 1	12 209	1	9 248	3	Very thin, falling hair, dermatitis. Death from cachexia. No pancreatic tissue found.
6	12.7 8 1	10 R	8 367	30	Carb. 1	8 253	7	22 295	22	Tol. 1	5 188	2	17 327	27	Thin, living.
7	8.9 7.1	8 R	16 383	16	Carb. 1	8 245	5								Died after 11 gm. of drug in 11 days. No pancreatic tissue found.
8	9.7 7.8	8 R	10 324	49	Carb. 1	13 280	22	8 322	38	Tol. 1	19 298	26	11 326	31	Living
9	12 4 10 0	16 R	20 366	33	Carb. 1	10 216	4								Died after 13 gm. of drug in 13 days. No recognizable pancreatic tissue found.

P = protamine zinc insulin R = regular insulin

Data for all periods except the initial control are calculated by omitting the first 3 days. Thus, the actual duration of these periods was 3 days longer than indicated.

* Single determination.

† Figures in this position indicate duration of period in days.

phyrins, and occult blood were negative. Post-mortem examination revealed a light orange staining of the fat depots The stomach and intestine contained large amounts of dark red material that did not appear to be blood, although specific tests were not done. There was no sign of bleeding from the mucosal surfaces. The liver and kidneys were grossly and microscopically normal except for occasional nodular glomerular lesions that seemed unrelated to drug therapy. No recognizable pancreatic tissue was found.

Dog 9 was found dead in its cage 3 days after completing a 13-day course of carbutamide therapy at a dosage rate of 1 gm. per day. The urine had become orange-brown, but the animal had seemed to be in excellent health the evening before death. At autopsy there was no staining of the fat depots. The liver and kidneys were grossly and microscopically normal, except for the presence of considerable protein within the glomerular capsules and in some tubules, presumably agonal. The small bowel was heavily infested with roundworms, but the intestinal contents were of normal color and there was no evidence of mucosal hemorrhage. The stomach was distended with undigested food, suggesting that the dog had died soon after eating and receiving insulin. The cause of death was uncertain, but may have been hypoglycemia. No recognizable pancreatic tissue was found.

The response of blood and urinary glucose to treatment with the drugs is shown in TABLE 1 and FIGURES 1 to 3. Mean values in experimental and recovery periods, but not in the initial control periods, are calculated by omitting the first three days, in order to avoid the influence of the preceding period and a possible lag in the full effect of the drug.

It is evident that, in all animals receiving insulin, carbutamide and tolbutamide produced a significant reduction in glycosuria and, to a lesser extent, the level of fasting blood sugar. Although the anorexia that developed in Dog 2 while receiving carbutamide doubtless played some part in the diminished glycosuria, the data show a marked drop in glucose excretion while food intake was still normal. The first experiments on Dog 5 were carried out without insulin, and in this circumstance carbutamide had no effect. In later experiments, when insulin was given, the animal responded well to both drugs.

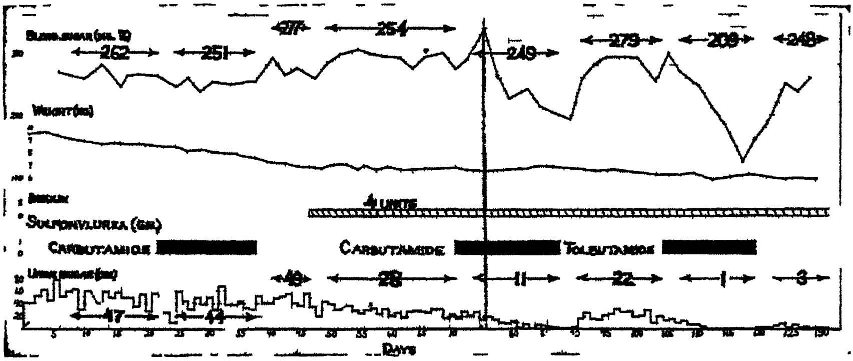

FIGURE 1. Dog HA-5T, totally depancreatized 19 days. In this and succeeding charts, figures between horizontal arrows represent means for the periods indicated.

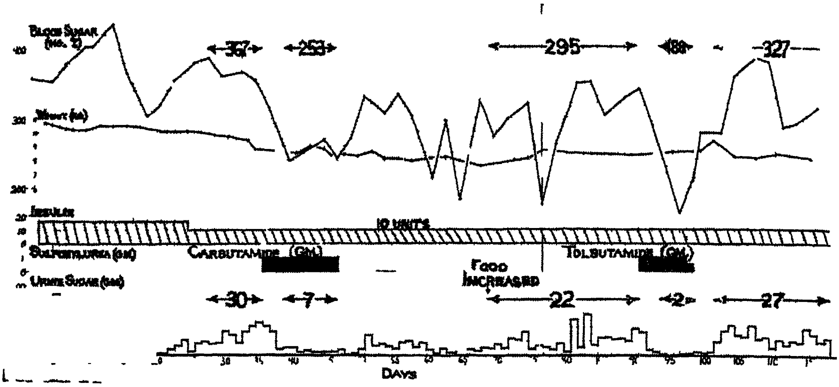

FIGURE 2. Dog HA 6-T, totally depancreatized 24 days

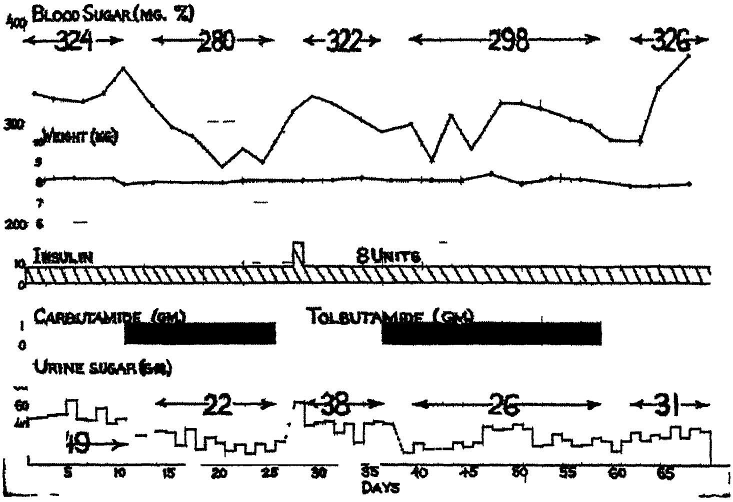

FIGURE 3. Dog HA-8-T, totally depancreatized 63 days.

The fact that this dog survived for more than two weeks in the initial control period without insulin, and in apparently good health, raised the question of whether pancreatectomy had been complete. Failure to find any pancreatic tissue at autopsy renders this case puzzling, and the possibility that such tissue may have been missed must be considered.

Discussion

The ability of carbutamide and tolbutamide to diminish fasting blood sugar levels and glycosuria in totally depancreatized dogs maintained with sub-optimal doses of insulin demonstrates that these effects can take place without the intervention of the pancreas. This is not necessarily incompatible with experiments indicating that insulin secretion is stimulated when the pancreas is intact;[7-9] nor does it eliminate the possibility that the α cells, when present. are suppressed, although the evidence originally implicating them has been largely refuted.[10, 11]

The data permit no decision between the alternative conclusions (1) that injected insulin is potentiated or (2) that glucose disposal is accelerated or its release retarded in tissues for which a certain minimum of insulin is necessary. There is some evidence from other sources against the first alternative. If the activity of insulin were augmented by the drugs (or for that matter if its secretion were enhanced in the presence of the pancreas), it would be expected that its known effects, in addition to hypoglycemia, would be intensified. Yet the sulfonylureas reportedly do not elevate blood lactate or pyruvate,[12-14] alter blood sugar or phosphorus curves after glucose loading,[12-15, 17] or decrease nitrogen excretion[12, 14, 16, 17] in the diabetic patient; they do not accelerate the oxidation of C^{14}-labeled glucose, as measured by recovery of $C^{14}O_2$ in eviscerated, nephrectomized rabbits;[18] and they do not increase the uptake of glucose by the rat diaphragm[19, 20]—all of which are accomplished by insulin.

If a primary tissue effect should be established, the most likely site would be the liver, since the compounds apparently do not alter carbohydrate metabolism in the periphery. An influence on the endocrine system exclusive of the pancreas seems unlikely, for it has been shown that sulfonamide hypoglycemia occurs in hypofunction or absence of the pituitary and adrenals;[2, 11, 12, 14, 17, 21-24] and the inhibition of thyroid activity noted by some[14, 17, 25-28] is slight and probably insignificant in the present connection.

The results reported here seemingly are inconsistent with the generally observed failure of these compounds to lower glucose levels in the juvenile diabetic receiving reduced amounts of insulin.[25, 29-31]

Summary

Carbutamide and tolbutamide regularly decreased levels of blood and urinary glucose in totally depancreatized dogs maintained with small doses of insulin.

Three of six animals died during or soon after administration of carbutamide, two of them with toxic symptoms.

The preponderance of available evidence indicates that, except for hypoglycemia and glycogen storage, the known effects of insulin are not duplicated by sulfonamide therapy, suggesting that the lowering of blood sugar observed in these experiments is attributable to tissue effects, probably in the liver, rather than to potentiation of injected insulin.

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STUDIES CONCERNING THE ROLE OF THE LIVER IN THE HYPOGLYCEMIC RESPONSE OF ANIMALS TO TOLBUTAMIDE

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Introduction

The hypoglycemic activity of certain sulfonylureas in intact animals and normal humans, as well as in a large number of diabetics is well documented.[1-5] The mechanism responsible for this action is still not understood, although several concepts have been advanced to explain it.

The original observation[6] of α-cell destruction by these drugs, which led to the conclusion that they act by decreasing glucagon secretion, has not been confirmed.[7-9] Since they were found ineffective in severe alloxan diabetes,[10-12] it seems unlikely that glucagon inhibition is an important factor in the hypoglycemic action.

A second theory, proposed by Mirsky *et al.*[13] and by Williams,[14] suggested that tolbutamide (Orinase*) and related drugs inhibited insulinase destruction of endogenous insulin. Later information[15, 16] indicated that this was probably not the principal action of these drugs in producing hypoglycemia.

The sulfonylureas have been found to have effects on the liver that might be expected to result in hypoglycemia. At high concentrations they inhibit glucose-6-phosphatase[17, 18] and phosphorylase reactivation[19] *in vitro*. In addition, a decrease in glucose-6-phosphatase activity is found in the livers taken from treated animals.[17, 20] Others have reported that tolbutamide and related drugs inhibit the conversion of fructose to glucose in the liver, suggesting an inhibition of gluconeogenesis.[21, 22] Supporting the liver site of action are the observations that showed that tolbutamide at hypoglycemic doses increases liver glycogen content in fasted intact animals.[12, 23] However, the role of inhibition of liver enzymes in the effects of these drugs on blood sugar is still uncertain, since nonhypoglycemic compounds can inhibit phosphorylase reactivation.[19] Glucose-6-phosphatase activity is also depressed in livers from insulin-treated animals;[17] the times of depression of blood sugar and of glucose-6-phosphatase do not correlate,[17] and no effect on blood sugar is observed in severe alloxan diabetic or depancreatized animals.[10-12, 16] Further, the concentrations required for *in vitro* inhibition of liver enzymes are considerably above the blood concentration required for an effect on blood sugar.

The early work of Loubatières suggested that the activity of the sulfonylureas on blood sugar was achieved by stimulation of insulin secretion by the β cells.[24] This theory has found recent support in a cross-circulation experiment[25] and in those experiments involving the infusion of small doses of the drug into the pancreatic artery.[26] It has been observed also that the number of pancreatic β cells is increased after treatment with one of these compounds.[27] Tolbutamide increases the utilization of labeled C^{14}-glucose[28, 29] in intact rats, which shows it to be similar in action to insulin. Opposing the β-cell stimulation concept are differences observed in the action of tolbutamide and insulin on blood pyruvate[21, 22] and on liver and muscle glycogen deposition.[23]

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* Registered trade-mark of The Upjohn Company for 1-butyl-3-*p*-tolylsulfonylurea.

In this paper we shall describe some experiments designed to study the relative importance of the pancreas and liver in the hypoglycemic response of animals to tolbutamide. This has involved principally a study of the effects of tolbutamide on blood sugar in hepatectomized animals and a further comparison of the effect of insulin and tolbutamide on blood sugar, and on muscle and liver glycogen when given under various experimental procedures and metabolic conditions.

TABLE 1
EFFECTS OF TOLBUTAMIDE ON BLOOD SUGAR OF EVISCERATED RATS INFUSED I.V. WITH INSULIN AND GLUCOSE

No rats	Treatment	Blood sugar (mg. %)
5	Insulin, 0.5–0.6 units/kg. hr. + glucose, 440 mg./kg. hr.	82.5
6	Insulin + glucose + tolbutamide, 250 mg./kg.	84.5

Methods and Results

Effect of tolbutamide in eviscerated rats. Male rats obtained from the Upjohn colony were eviscerated following the method of Ingle and Griffith[30] after a 24-hr. fast while under anesthesia induced by intraperitoneal injection of cyclopal sodium (32 mg./kg.)* and a subcutaneous injection of phenobarbital sodium (132 mg./kg.). The adrenals and kidneys were left intact. Following the operation the animals were immediately connected to a constant-infusion apparatus via the saphenous vein and were infused with 0.5 to 0.6 units of crystalline insulin† and 440 mg. of glucose per kg. of body weight per hour. One half of these animals received a 250 mg./kg. dosage of sodium tolbutamide subcutaneously. Exactly 4 hr. later they were bled from the jugular vein, and blood sugars were determined in duplicate by the ferricyanide procedure.[31] The temperature during the infusion period was maintained at 26.5 ± 0.5° C.

The average blood sugar of the eviscerated rats receiving insulin, glucose, and tolbutamide was 85 mg. per cent, as compared to 83 mg. per cent in those that received only the glucose and insulin (TABLE 1). Tolbutamide was therefore ineffective in stimulating glucose utilization in the absence of two important blood sugar-regulating organs, the liver and the pancreas.

Effect of tolbutamide in alloxan diabetic rats. In male rats obtained from the Upjohn colony and weighing 300 to 350 gm., alloxan diabetes was produced by intravenous administration of alloxan at a dosage of 42 mg./kg. in one rapid injection. These animals had been diabetic 4 to 6 months prior to this experiment. They were kept in regular wire cages on ad libitum Purina Laboratory Chow and tap water until ready for the metabolic experiments, at which time they were put into metabolism cages and cup-fed 13 cc. of liquid diet[32] twice a day. Daily urinary glucose determinations were made by the ferricyanide method[31] for each animal until sugar excretion became relatively constant for 7 consecutive days. After this control period, tolbutamide was administered orally by stomach tube immediately prior to each feeding for 1-week periods, a week being allowed to elapse between each dosage regimen. Each value given represents the average of 7 daily determinations.

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* 5-(1-cyclopenten-2-yl)-5-allylbarbituric acid sodium.

† Iletin, Eli Lilly and Company, Indianapolis, Ind.

An experiment was also carried out to determine whether tolbutamide potentiated the effects of exogenous insulin on urinary glucose excretion in alloxan diabetic animals that had been previously found not to respond to tolbutamide. These animals were kept in metabolism cages and were adapted to force-feeding of 13 cc. of liquid diet twice a day. After 1 week of stabilizing on the force-feeding, each animal's urinary glucose was determined daily and, after the excretion became relatively constant for 7 days, therapy was initiated. All animals were injected with 1 unit of crystalline insulin 2 times per day, and one half of these animals received tolbutamide (375 mg./kg.) twice a day with their food.

Severely alloxan diabetic rats did not respond uniformly to tolbutamide with a decrease in urinary glucose excretion during three 1-week courses of treatment (TABLE 2). The slight changes in glucose excretion seen for some of these animals were not significant, since day-to-day variations could have been of that magnitude. It was of interest that the urine sugar of Rat No. 7, which was 3300 mg. for 24 hr. prior to tolbutamide treatment, was depressed

TABLE 2
EFFECTS OF TOLBUTAMIDE ON URINARY GLUCOSE EXCRETION BY ALLOXAN DIABETIC RATS

Rat No.	Body weight (gm.)	Excretion prior to treatment*	Excretion on 125 mg. drug*	Excretion after drug withdrawal*	Excretion on 250 mg. drug*	Excretion after drug withdrawal*	Excretion on 350 mg. drug*	Excretion after drug withdrawal*
1	295	5.6 gm.	5.8 gm.	6.2 gm.	5.6 gm.	6.6 gm.	6.4 gm.	6.2 gm.
2	330	7.1	6.5	7.3	6.9	7.3	6.3	6.0
3	272	5.6	5.0	6.1	5.3	6.2	4.9	6.3
4	245	6.4	6.0	7.0	6.1	6.4	5.1	6.3
5	292	5.1	5.2	6.7	6.3	6.8	5.8	5.8
6	291	5.1	5.3	5.1	5.6	6.3	5.1	5.7
7	354	3.3	1.7	4.3	—	—	—	—
Average 1–6		5.8	5.6	6.4	6.0	6.6	5.6	6.0

* Mean of 7 daily determinations.

TABLE 3

EFFECTS OF INSULIN PLUS TOLBUTAMIDE ON URINARY GLUCOSE EXCRETION OF ALLOXAN DIABETIC RATS

Treatment	Animals	Pretreatment urinary glucose*	Urinary glucose while on treatment†
2.0 units crystalline insulin	4	4.3 gm	2.4 gm
2 units insulin + 375 mg tolbutamide/kg	4	4.8 gm	2.3 gm

* Average of 7 daily determinations

† Average of 4 daily determinations

by about 50 per cent during treatment. This animal, however, was considered mildly diabetic and presumably still possessed functional β cells and the ability to secrete insulin.

When 2 units of crystalline insulin per day were given to alloxan diabetic animals that did not respond previously to tolbutamide, the glucose excretion values dropped approximately 50 per cent. When tolbutamide was given simultaneously with the insulin in this type of animal, there was no further drop in glucose excretion (TABLE 3). It can therefore be said that under these conditions tolbutamide does not potentiate the activity of exogenous insulin in the alloxan diabetic animal.

Effects of tolbutamide on blood sugars of glucose-infused hepatectomized rats and dogs. Male rats obtained from the Upjohn colony, weighing 325 to 350 gm., were prepared for hepatectomy by partially ligating (to the size of a 19-gauge hypodermic needle) the hepatic portal vein as close as possible to the liver and the inferior vena cava between the right kidney and liver. After preliminary experiments it was found that 4 weeks was sufficient for the establishment of sufficient collateral circulation for hepatectomy to be successfully accomplished. The animals were anesthetized by the intraperitoneal injection of cyclopal sodium (32 mg./kg.) and a subcutaneous injection of phenobarbital sodium (132 mg./kg.). The operation was carried out through a midventral incision following a 24-hr. fast, with care being taken not to sever the enlarged abdominal veins. Following the operation the animals were immediately connected to a constant-infusion apparatus via the saphenous vein and were infused with 250 or 125 mg. of glucose per kg. per hr. in a volume of 0.9 cc. of water per hr. The vehicle-treated animals and the animals that received 400 mg./kg. dosages of subcutaneously injected sodium tolbutamide were paired exactly on a body-weight basis. The body weight at the time of hepatectomy ranged from 375 to 450 gm. During the infusion period the temperature was maintained at 26.5 ± 0.5° C. At the end of 4 hours the animals were bled from the jugular vein, and blood sugar determinations were made in duplicate by the ferricyanide procedure.[31] Animals that became cyanotic in the posterior musculature during the experiment were discarded, since it was considered that they lacked sufficient collateral circulation established from the inferior vena cava. The success of the

TABLE 4

EFFECTS OF TOLBUTAMIDE ON BLOOD SUGARS OF HEPATECTOMIZED RATS

Treatment	Blood sugar (mg. %)	
	Saline	Tolbutamide (400 mg./kg.)
Glucose I.V. (25 mg. 100 gm. body weight/hr.)	300	265
	289	232
	235	199
Glucose I.V. (12.5 mg./100 gm. body weight/hr.)	84	60
	100	40
	121	75
	125	106
	135	112
	64	45

hepatic portal ligation in the induction of collateral circulation was determined by whether the digestive tract became cyanotic at the time of hepatectomy after the hepatic portal vein was completely ligated.

The dogs used for the hepatectomy experiments were healthy, young, mature mongrels of both sexes, weighing 19 to 29 kg. Following a 24-hr. fast they were anesthetized by intravenous injection of 250 mg. kg. doses of barbital sodium[*] and 20 mg./kg. doses of pentothal sodium.[†] Hepatectomy was accomplished in a one-stage operation following the method of Markowitz *et al.*[33] This involved cannulation of the inferior vena cava through the liver and diaphragm, formation of an Eck's fistula and then removal of the liver following ligation of the hepatic artery, portal vein, and bile duct. Blood samples were obtained from the jugular and glucose determined by the Folin-Wu method.[34] The animals were infused via the femoral vein with 125 mg./kg./hr. of glucose in 17.5 cc. of water. Immediately after the operation sodium tolbutamide at 50 or 100 mg./kg. was given intraperitoneally to 3 of these hepatectomized animals. Other hepatectomized dogs received only glucose and served as controls. The data reported here were obtained only from those animals that remained in relatively good condition for the duration of the experiment. Blood sugar values for control and tolbutamide-treated laparotomized dogs under identical anesthesia as that used in hepatectomy were included for comparative purposes. These dogs did not receive glucose.

Subcutaneously administered tolbutamide in hepatectomized rats infused with glucose at 125 mg./kg. consistently decreased the blood sugar (TABLE 4). It is interesting to note that when glucose was given at 250 mg./kg./hr. the tolbutamide effect was not as great on a percentage basis as with the lower glucose load. However, it should be noted that the actual depression on a milligram per cent basis was slightly greater with the larger glucose load.

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[*] Diethylbarbituric acid.

[†] 5-Ethyl-5-(1-methylbutyl)-2-thiobarbiturate.

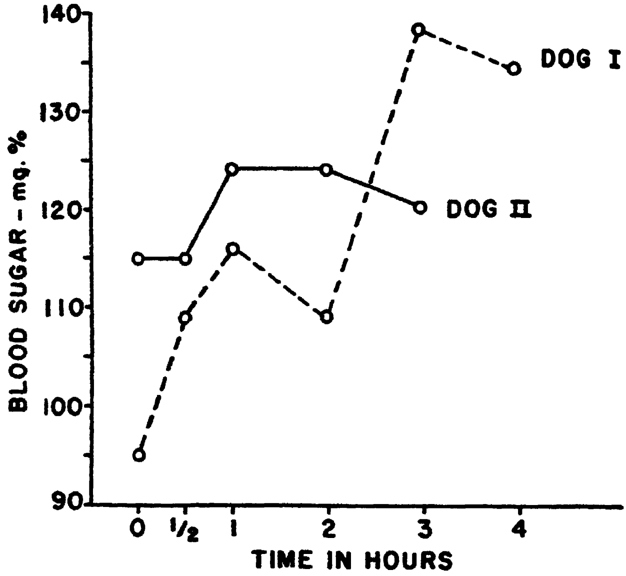

Figure 1. Blood sugars of hepatectomized dogs infused I.V. with glucose at 125 mg./kg./hr

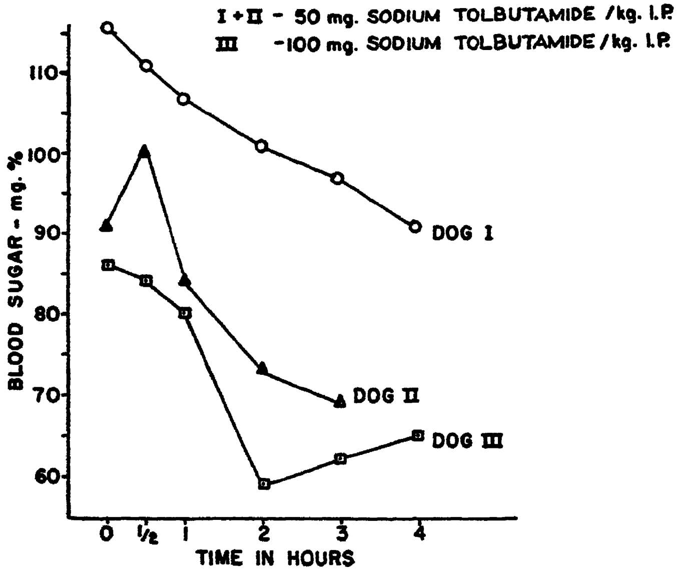

Figure 2. Effect of intraperitoneally administered sodium tolbutamide on the blood sugar of hepatectomized dogs infused I.V. with glucose at 125 mg./kg./hr.

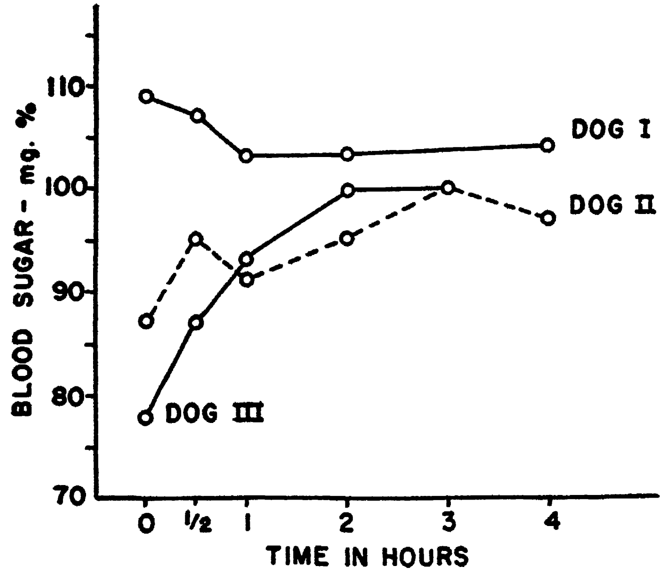

FIGURE 3. Blood sugar of laparotomized anesthetized intact dogs.

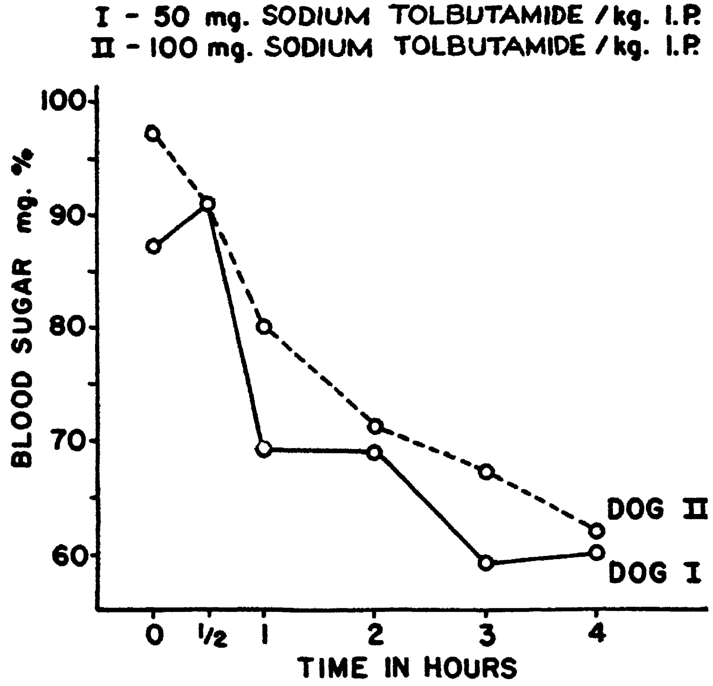

FIGURE 4. Effects of intraperitoneally administered sodium tolbutamide on the blood sugar of laparotomized anesthetized intact dogs.

The activity of tolbutamide in hepatectomized rats was confirmed in hepatectomized dogs. From FIGURE 1 we see that dogs that were infused with intravenous glucose at 125 mg./kg./hr. had a slight elevation of blood sugar Two similarly treated animals that received tolbutamide had a significant depression of blood sugar (FIGURE 2). It is interesting that the result in hepatectomized animals was of approximately the same magnitude as that obtained in laparotomized, anesthetized intact dogs that received no glucose infusion (FIGURES 3 AND 4). It is evident from these results on hepatectomized rats and dogs that tolbutamide is effective in animals without a liver, but with a functional pancreas.

Analysis of effects of tolbutamide on muscle and liver glycogen in relation to the hypoglycemic response. The intact rats used in this study were males weighing 140 to 160 gm. Adrenalectomies were performed at a body weight of 140 to 160 gm., and these rats were maintained on 1 per cent sodium chloride drink and Purina Laboratory Chow 10 to 16 days prior to use. All animals were fasted 24 hr. prior to treatment. The sugars were administered intraperitoneally, tolbutamide orally, and insulin and hydrocortisone subcutaneously. Sucrose was used as a control for the glucose treatment. Glucose and the drugs were always given simultaneously. Blood samples were

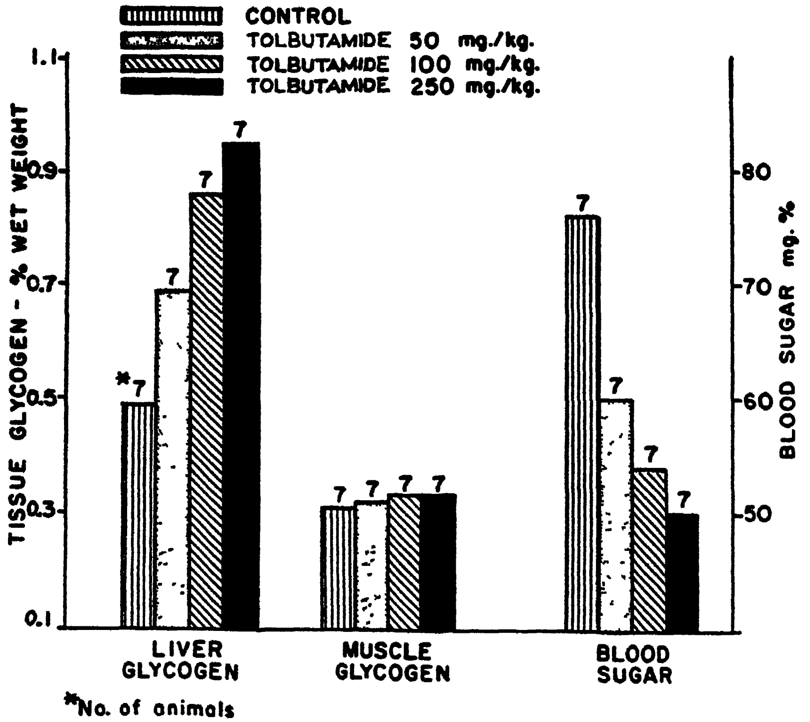

FIGURE 5 Effects of oral tolbutamide on the liver and muscle (diaphragm) glycogen and blood sugar of fasted intact rats 2 hr after treatment.

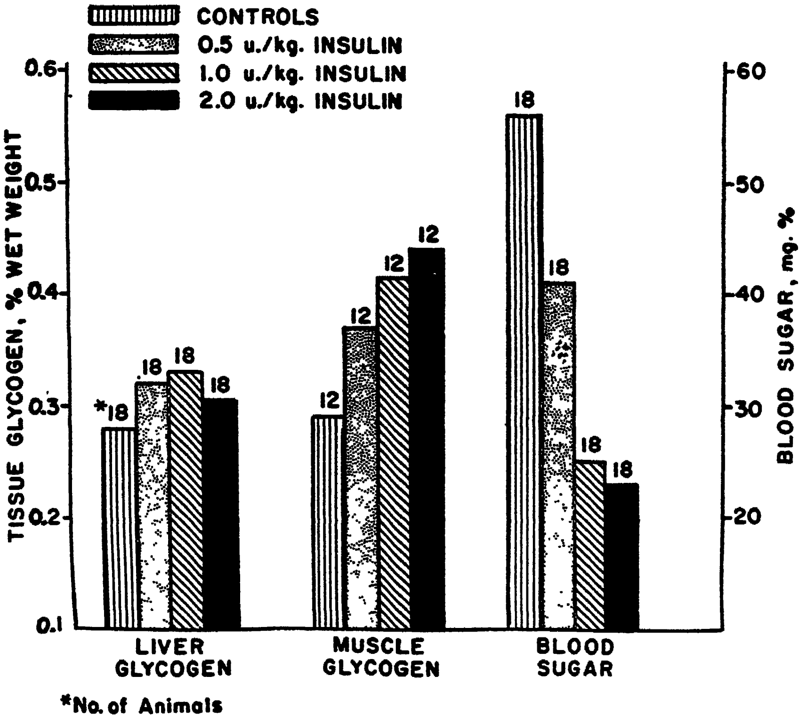

FIGURE 6. Effects of subcutaneously administered glucagon-free insulin on the liver and muscle (diaphragm) glycogen and blood sugar of fasted intact rats 2 hr. after treatment.

obtained from the vena cava while the animals were under cyclopal sodium anesthesia, and sugar was estimated by the Folin-Wu method.[34] Tissue glycogen was estimated by the anthrone method[35] on tissues removed immediately following bleeding. The insulin* used in this study was glucagon-free and had a low zinc content.

When tolbutamide was given to intact fasted rats there occurred an increase in liver glycogen (FIGURE 5) and a decrease in blood sugar, with no change in muscle glycogen. Glucagon-free insulin at doses that produced a depression of blood sugar did not consistently change liver glycogen deposition in intact animals, although there was an increase in muscle glycogen (FIGURE 6).

Tolbutamide was found not to alter the liver glycogen or muscle glycogen in adrenalectomized animals, although there was a striking depression of blood sugar (FIGURE 7). When liver glycogen deposition was produced by glucose in adrenalectomized animals, both tolbutamide and glucagon-free insulin inhibited the increase in glycogen and also depressed the blood sugar (FIGURES 8 AND 9). It was seen also that tolbutamide did not influence the

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* The glucagon-free insulin was kindly supplied by Otto Behrens, Eli Lilly Company, Indianapolis, Ind.

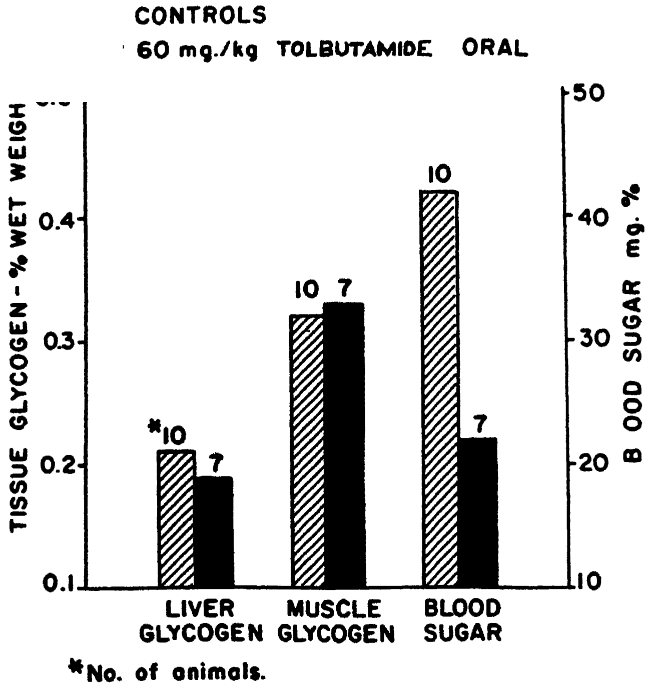

FIGURE 7. Effect of oral tolbutamide on the blood sugar and liver and muscle (diaphragm) glycogen of fasted adrenalectomized rats 3 hr. after treatment.

muscle glycogen, while the glucagon-free insulin consistently increased muscle glycogen.

Effects of constant infusion of small doses of insulin on muscle glycogen and blood sugar. Male rats of the Upjohn strain weighing between 220 and 240 gm. were anesthetized with intraperitoneally administered cyclopal sodium (32 mg./kg.) and with subcutaneously administered phenobarbital (132 mg./kg.). They were infused with an insulin solution so that each animal received insulin (Iletin), 1 unit/kg. in 0.5 cc. of saline over a 4-hr. period via the saphenous vein. During the infusion period the temperature was maintained at 26 ± 0.5° C. At the end of 4 hr. the animals were bled via the vena cava, the blood was oxalated immediately, and sugars were estimated by the Folin-Wu method.[34] Following withdrawal of the blood, the diaphragms were removed and glycogen determinations were made by the anthrone method.[35] The data reported here are pooled from 2 different experiments. It is readily apparent (TABLE 5) that the 1 unit/kg. dosage of insulin, infused over the 4-hr. period, produced depression of blood sugar with no significant change in muscle glycogen.

Discussion

The failure of tolbutamide to influence glucose utilization in the eviscerated rat is in agreement with other reports [16, 36] and implies that the liver and/or

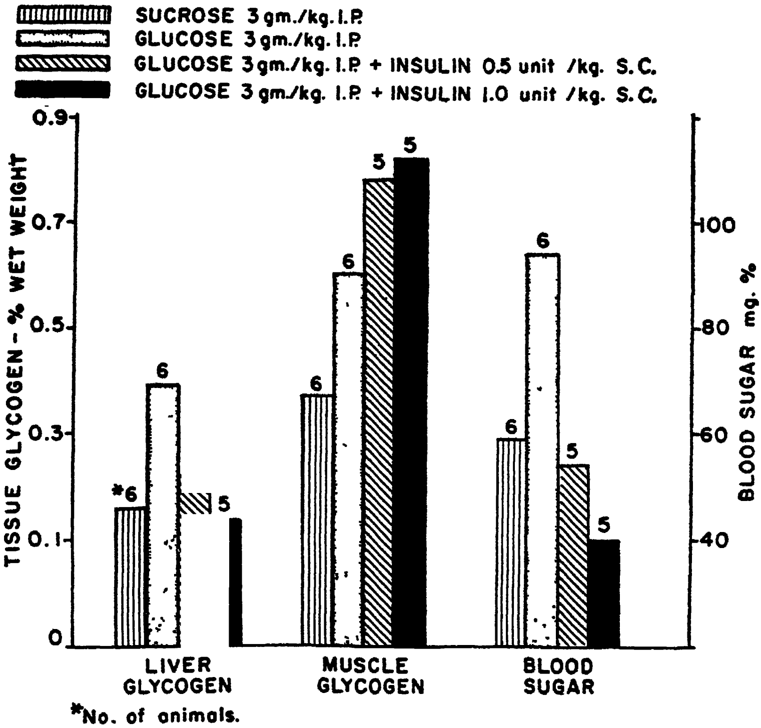

FIGURE 8. Effects of subcutaneously administered glucagon-free insulin on the blood sugar and liver and muscle (diaphragm) glycogen of intraperitoneal glucose-treated fasted adrenalectomized rats 2 hr. after treatment.

the pancreas is required as a target organ for the hypoglycemic response of tolbutamide to be manifested. The inability of severe alloxan diabetic animals to respond to this drug[10-12] suggests that functional β cells must be present to obtain a depression of blood sugar. The fact that hepatectomized dogs and rats that possess a pancreas with an intact blood supply respond to tolbutamide shows that the liver is not essential and minimizes the necessity of inhibiting liver insulinase or enzymes involved in hepatic glucose release in

TABLE 5
EFFECT OF CONSTANT INFUSION OF INSULIN ON BLOOD SUGAR AND MUSCLE (DIAPHRAGM) GLYCOGEN

No. rats	Treatment	Blood sugar (mg. %)	Muscle glycogen (% wet weight)
9	Saline (0.5 cc. 4 hr.)	60	0.31
12	Insulin (1.0 unit kg. /4 hr.)	44*	0.37

* Significant at 5 per cent level of confidence as tested by the t-test.

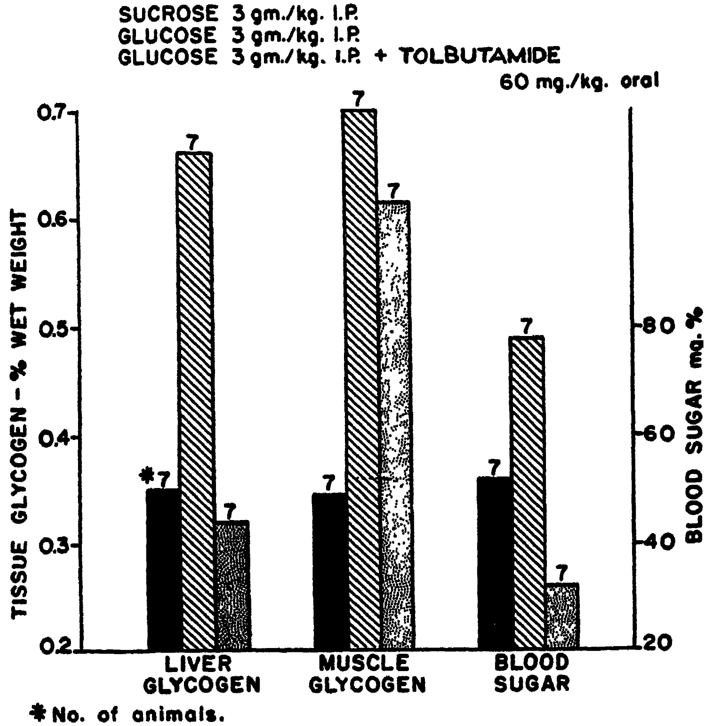

FIGURE 9. Effects of oral tolbutamide on the blood sugar and liver and muscle (diaphragm) glycogen of intraperitoneal glucose-treated fasted adrenalectomized rats 3 hr. after treatment.

order to obtain a response. The failure of tolbutamide to potentiate insulin action in alloxan diabetic animals also indicates a lack of insulinase inhibition. Since this sulfonylurea is ineffective in the presence of the liver and the absence of functional β cells, but does exert its effect in the absence of the liver and the presence of β cells, it seems apparent that the only organ absolutely necessary for the pharmacological effects of tolbutamide on blood sugar is the pancreas. These data support the concept that the principal action of this agent in producing a reduction in blood sugar is the stimulation of the secretion of insulin or some other hypoglycemic agent by the β cells of the pancreas.

The above results do not resolve certain differences observed between the action of insulin and tolbutamide. It has been reported[23] and confirmed in this paper that tolbutamide increases liver glycogen, but does not alter muscle glycogen in fasted intact animals, while glucagon-free insulin increases muscle glycogen with no change in liver glycogen. These differences might be interpreted to indicate that tolbutamide does not act in the same way as insulin and, therefore, has some action other than that of increasing insulin secretion that may be important in its hypoglycemic effects; for example, inhibition of enzymes of the liver, with a resultant decreased hepatic glucose output. The failure of tolbutamide to increase muscle glycogen suggests that it does not increase peripheral uptake and oxidation of glucose.

Data bearing on these differences were obtained in the studies on adrenalectomized rats. In these animals tolbutamide produced a striking depression of blood sugar with no change in liver glycogen. Therefore, this drug can produce a reduction of blood sugar with no increase in liver glycogen. This indicates that its effect on liver glycogen in intact animals is coincidental to the hypoglycemic action and is probably elicited through secondary mechanisms. The factors involved in the glycogen deposition effects seen in the fasted intact rat are not understood at this time, although it is possible that this is a result of adrenal stimulation. When adrenalectomized rats were given glucose to induce liver glycogen deposition, both tolbutamide and glucagon-free insulin inhibited the glycogen deposition. Therefore, the effect on glycogen storage depends on other existing metabolic conditions and, in this case, tolbutamide and insulin produce the same response, which is in agreement with the concept that tolbutamide increases insulin secretion.

The difference in effect of insulin and tolbutamide on muscle glycogen deposition can be used as evidence that this drug does not act via insulin secretion. This discrepancy could be due to the fact that insulin was administered by a single rapid injection, while the tolbutamide effects were prolonged and could have been causing a steady release of a small amount of insulin. This hypothesis is supported by the observation that constant intravenous infusion of a small dose of insulin can mimic tolbutamide by causing a significant depression of blood sugar with no change in muscle glycogen. These results suggest also that other observed differences between the action of tolbutamide and insulin could possibly be resolved by administering the insulin by constant infusion; for example, insulin increases pyruvate and tolbutamide does not, while tolbutamide increases liver glycogen in fasted intact rats, and insulin does not.

The results reported here and an increasing volume of published data strongly indicate that the principal activity of tolbutamide and other sulfonylureas is to stimulate the secretion of insulin or some other hypoglycemic agent by the β cells of the pancreas. However, under certain conditions, these drugs can produce other actions that are probably secondary to their hypoglycemic effect through stimulation of insulin secretion. The observations supporting this conclusion are as follows: (1) tolbutamide and related drugs are active in intact animals, while they are inactive in the severe alloxan diabetic and depancreatized animals and humans, and in juvenile human diabetics;[5, 10, 16, 22] (2) the hypoglycemic action is manifested in animals without a liver; (3) the difference previously observed between the activity of tolbutamide and insulin on muscle glycogen may be due to the method of insulin administration; (4) insulin and tolbutamide decrease glucose-induced liver glycogen in adrenalectomized rats; (5) tolbutamide can produce a depression of blood sugar without altering liver glycogen in adrenalectomized rats, showing that the liver glycogen and blood sugar effects can be separated; (6) both tolbutamide and insulin have been found to increase oxidation of C^{14}-labeled glucose;[28, 29] (7) infusion of hypoglycemic sulfonylureas into the pancreatic artery at doses that are inactive when perfused through other blood vessels produces a hypoglycemic response;[2, 26] (8) in cross-circulation experiments, blood from the pancreatic but not from the mesenteric vein of a sulfonylurea-treated donor causes a depression of blood sugar in the recipient animal,[25] and pancreatic vein blood from a treated normal donor can cause a hypoglycemia in alloxan diabetic recipient dogs;[2] (9) one of these drugs (carbutamide, BZ-55) increased the number of β cells of the pancreas in normal animals.[27]

Summary

Tolbutamide was ineffective in lowering the blood sugar of eviscerated rats or the urine sugar of severely alloxan diabetic rats, but was capable of depressing blood glucose in hepatectomized rats and dogs. In the adrenalectomized rat the effects of this drug on liver glycogen and blood sugar were not related, and it was suggested that the glycogen storage seen in the intact animals was due to secondary responses. Glucagon-free insulin and the sulfonylurea inhibited glucose-induced liver glycogen deposition in adrenalectomized rats; and the slow intravenous infusion of insulin was found to mimic tolbutamide in producing a decrease in blood sugar with no change in muscle glycogen. These observations, along with those of other investigators, have been interpreted to mean that tolbutamide lowers blood sugar by the stimulation of the secretion of insulin or some other hypoglycemic factor by the β cells of the pancreas.

Acknowledgment

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THE MECHANISM OF ACTION OF THE HYPOGLYCEMIC SULFONAMIDES: A CONCEPT BASED ON INVESTIGATIONS IN ANIMALS AND IN HUMAN BEINGS

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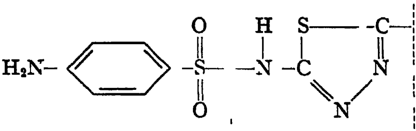
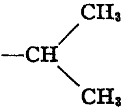
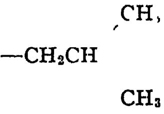
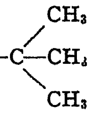
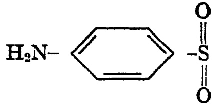
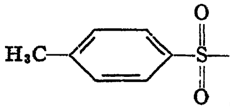
The majority of physiological or pharmacological substances are not characterized by a simple specific exclusive mechanism of action. In addition to the principal or preferential action there exist secondary effects which, depending upon experimental conditions or species, may become more prominent than the principal effect. Sometimes the secondary actions reinforce the primary one, and sometimes they are in opposition. Under certain circumstances the interrelationship between the primary and the

TABLE 1

(1) *Thiodiazole derivatives*		
		isopropyl (2254 RP)
" " —	$—CH_2CH_2CH_2CH_3$	butyl (2263 RP)
" " —	 $—CH_2CH(CH_3)_2$	isobutyl (2256 RP)
" " —	 $—C(CH_3)_3$	tertiary butyl (2259 RP)
" " —	$—CH_2CH_2CH_2CH_2CH_3$	amyl (2261 RP)
(2) *Urea derivatives*		
 -NHCONH—	$—CH_2CH_2CH_2CH_3$	butyl (BZ-55, carbutamide)
 —NHCONH—	$-CH_2CH_2CH_2CH_3$	butyl (D 860, tolbutamide)

secondary effects is so intricate that it is difficult to dissociate one from the other.

The hypoglycemic sulfonamides are no exception to this rule; they have both primary and secondary actions. The emergence of one or the other depends upon the type of experiment (acute or chronic). It is the object of the physiologist or the pharmacologist to analyze and, if possible, to dissociate the observed effects.

The data obtained in the last fourteen years permit us to present a plausible if incomplete concept concerning the mechanism of action of the sulfonamides.

To begin with, let us consider the pharmacological data. Two types of substances have been studied in animals and in human diabetics, the thiodiazole derivatives, of which the initial compound was the isopropyl (2254 RP, IPTD) and the urea series of which the original was carbutamide (BZ-55). Tolbutamide (D 860) is a variant of carbutamide. When one arranges in parallel the substances we have studied with the sulfonylureas, the similarities become evident (TABLE 1). The substitution of a methyl for the NH_2 group on the benzene ring does not modify either the pharmacological or the therapeutic activity.

We shall consider the mechanism of the hypoglycemic action and then the mechanism of the antidiabetic action of these materials. These two properties were recognized between 1942 and 1946 by Loubatières, and they have since been confirmed by numerous authors using both series of compounds. In the second portion of this paper we shall discuss how the ideas derived from animal experimentation can be extrapolated to the diabetic human.

Analysis of the Hypoglycemic Action of the Sulfonamides in Animals

One important point is immediately evident: the fundamental role of the pancreas. The pancreas is indispensable to the hypoglycemic action; this effect does not occur in the totally depancreatized dog (FIGURE 1).[1-3, 4a, 5] On the other hand, if one leaves only a small quantity of pancreatic tissue in the abdomen, the hypoglycemic action becomes manifest.[1-4] In the hypophysectomized depancreatized dog, which is sensitized to hypoglycemia, the sulfonamides do not lower the blood sugar.[4a, 4b, 4c] It is not the level of the blood sugar, but rather the presence of the pancreas that conditions the hypoglycemic response to the sulfonamides.

The endocrine glands other than the pancreas are not essential for the hypoglycemic effect; it can be achieved in the rat lacking the pituitary, adrenals, thyroid, parathyroids, and gonads (unpublished experiments). The liver itself is not absolutely essential, since a certain degree of hypoglycemia can be produced after acute hepatectomy.[9a, 9b] In one experiment we were able to show that the hypoglycemic action of 2254 RP or of carbutamide was obtainable in the same dog, which was successively deprived of adrenals, thyroid and parathyroids, gonads, and pituitary (FIGURE 2). In this animal, during the course of postsulfonamide hypoglycemia, pancreatectomy interrupted the fall in blood sugar, and diabetes supervened.[10, 11]

The ablation of some endocrine glands increases the hypoglycemic action.

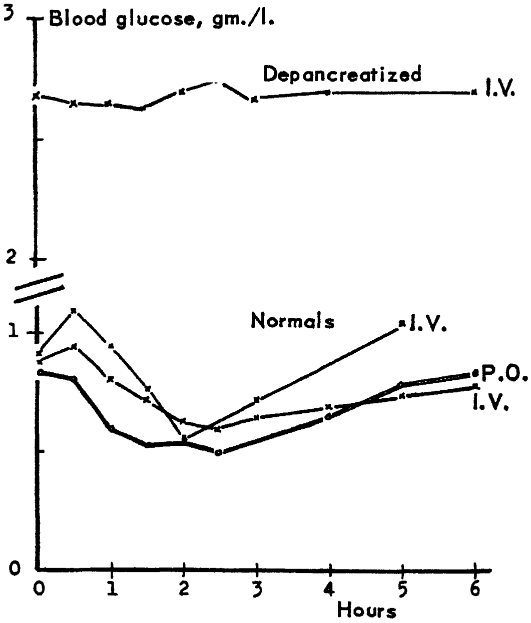


FIGURE 1. Hypoglycemic action of 2254 RP in the normal dog. Dose: 0.4 gm per kg. by mouth (P.O.) or by vein (I.V.). Note the lack of effect in the depancreatized animal (upper curve).

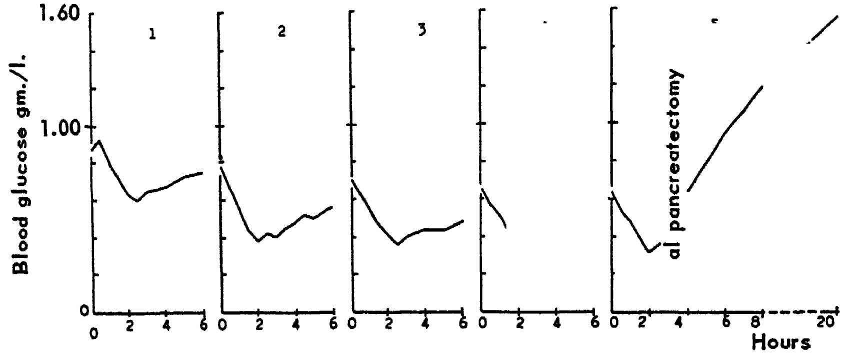


FIGURE 2. Effects of 2254 RP, 0.2 gm. kg. I.V., on the blood sugar of a dog subjected successively to various procedures: (1) normal control; (2) after bilateral adrenalectomy; (3) after subsequent thyroparathyroidectomy and gonadectomy; (4) after total hypophysectomy; and (5) after total pancreatectomy. Note the immediate interruption of the hypoglycemic effect of the drug by total pancreatectomy.

This is the case after hypophysectomy and especially after adrenalectomy.[4a-4c, 12-14] The toxic action of the sulfonamide and the degree and duration of the hypoglycemia increase after adrenalectomy. The administration of cortical steroids or of epinephrine protects the animals against the hypoglycemic action.[4a-4c, 12]

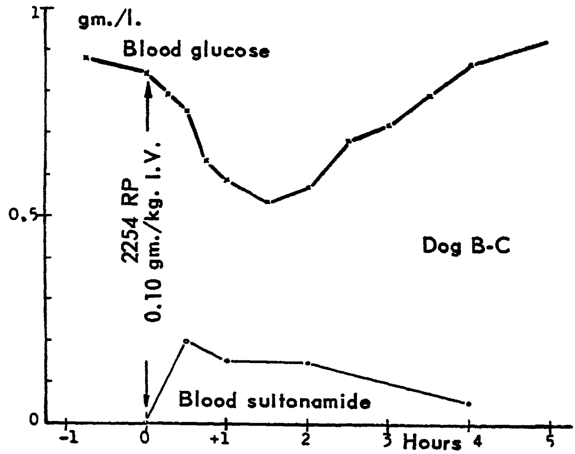


FIGURE 3. Hypoglycemic action of 2254 RP, 0.1 gm./kg., given intravenously in a dog under chloralose anesthesia. This animal was previously hypophysectomized, the vagi were cut, the carotids ligated, and the portion of the CNS anterior to the corpora quadrigemina was surgically removed.

Since the pancreas is implicated in the hypoglycemic action one must suppose that the sulfonamides activate the release of insulin from the islets of Langerhans, and one must study the mechanism of such release.

The higher nervous centers do not seem to take part in this action.[1, 2, 3] In the dog we have destroyed the brain, from the frontal lobes to the posterior limit of the corpora quadrigemina. We then destroyed the midbrain, removed the pituitary, sectioned the vagi in the neck, and ligated the carotids (FIGURE 3). Despite these procedures, postsulfonamide hypoglycemia was still produced.[15, 16] A nervous pathway is therefore not involved.

On the other hand, a small dose of a sulfonamide injected directly into the arterial supply of the pancreas produces hypoglycemia, even though the blood sulfonamide level is low.[1-3, 17] Therefore, the excitation of the insulin-secreting cells occurs by a humoral pathway, and the sulfonamide itself is the agent directly responsible.

It is possible that a local effect on blood vessels may contribute to the liberation of insulin, because there is an increased hyperemia of the pancreas after the administration of the drugs (unpublished experiments). The importance of this phenomenon is now under study in our laboratory.

The β cells of the islets seem to be the preferential site of action of these drugs. Histological and biochemical data are in favor of such a concept. The sulfonamides cause a relative degranulation of the β cells in acute or chronic experiments.[18-21] After chronic administration, there is hypertrophy of the islet system,[3, 22] new formation of β cells at the expense of the cells of the acini and of the ducts,[5, 18, 24] and sometimes appearance of mitotic figures.[18, 21] We think that these are signs of stimulation and of persistence of stimulation of the β cells. The sulfonamides produce little if any lowering of the blood sugar in severe alloxan or pituitary diabetes.[5, 16, 25, 26] On the other hand, in less intense diabetes, following the administration of alloxan, growth hormone, or cortisone, the sulfonamides produce hypoglycemia.[3, 5, 25-29] It appears, therefore, that the hypoglycemic response depends upon the number of functioning β cells spared by the diabetogenic substance. According to some authors the sulfonylureas lower the insulin content of the pancreas.[30]

The hypothesis of destructive action of the sulfonamides on the α cells, promulgated first by a group of German authors,[25, 31-34] has lost much of its validity, if one judges by recent publications.[19, 35] According to this concept, the sulfonamides damage the α cells to such a degree that the $\alpha:\beta$ ratio that exists in diabetes is compensated for by destruction of the α cells. This theory does not take into account the fact that glucagon has not been shown to be an anti-insulin substance under all conditions. It also does not take into consideration the experimental data that show that the sulfonamide compounds are inactive in severe alloxan diabetes, in which the α cells are preserved. Some of the above-mentioned authors have modified their initial viewpoint and admit that the sulfonylureas act by stimulating the β cells.[19, 35]

It is not impossible, however, that the sulfonamides act in some manner to stimulate the α cells and to liberate locally some glucagon(?), which in turn facilitates the liberation of insulin. This is in accord with the fact that in treated animals the glucagon content of the pancreas is not less than in normals.[36] Such a phenomenon is consistent with the fact that in mild alloxan diabetes the sulfonamides *are* hypoglycemic.

The hyperglycemic action of glucagon does not seem to be significantly affected by the drugs.[26, 36, 37a, 37b] However, in *in vitro* experiments using hepatic tissues and very high levels of sulfonamides, an inhibition of glucagon action was obtained.[36]

The mechanism of the action of these drugs on the β cells is unknown, but several hypotheses may be advanced. We may be dealing with a stimulation of insulin secretion, a liberation of insulin from granules that retain the hormone in the cell, an activation of insulin precursors, or an increased entry of insulin molecules into the circulation by reason of increased vascularity. One must remember, above all, that we are ignorant of the intimate physiological mechanisms concerned with the elaboration and secretion of endogenous insulin itself.

By means of cross-circulation experiments, one can detect the passage of a hypoglycemic substance liberated by the sulfonamides.[2, 3, 39]

It is also possible to show this by more simple experiments, for example by the injection of small quantities of the drug into the artery of the uncinate portion of the gland or into the canal of Wirsung.[1, 2, 3, 17] We do not know whether the properties of the endogenous insulin that is liberated are identical to those of insulin obtained by extraction. This endogenous insulin would be liberated normally into the portal system and carried to the liver, which probably increases the efficiency and activity of the hormone.

It is possible to show in the normal and in the depancreatized dog a curious property of these sulfonamides—a potentiating effect on exogenous insulin

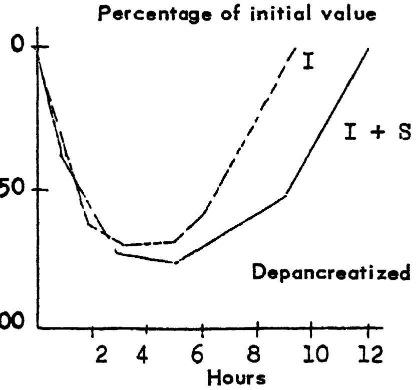


FIGURE 4. Hypoglycemic action of insulin (I) and of insulin plus 2254 RP (I + S) in a depancreatized dog. Dosage: insulin, 0.5 units kg. S.C.; 2254 RP, 0.25 gm. kg. given I.V. immediately before the insulin.

(FIGURE 4).[40, 41] This action does not require the presence of the pancreas, but does require exogenous insulin. We have recently shown that it is possible to introduce a small quantity of active insulin into the blood stream of a normal or depancreatized dog by giving the animal, by mouth, a solution of glycerol containing a very large dose of insulin (10 units per kg. body weight). The administration of 2254 RP, carbutamide, or tolbutamide potentiates the effects of the very small quantity of insulin that has been absorbed (FIGURE 5).[76]

These experiments show that the portal route is favorable to the action of insulin (which then acts directly on the liver) and also favors the potentiation by sulfonamides. These data are interesting from many points of view, especially with regard to the suggestion that these substances inhibit insulinase.[42, 43, 44] We are working with this program actively now, and it

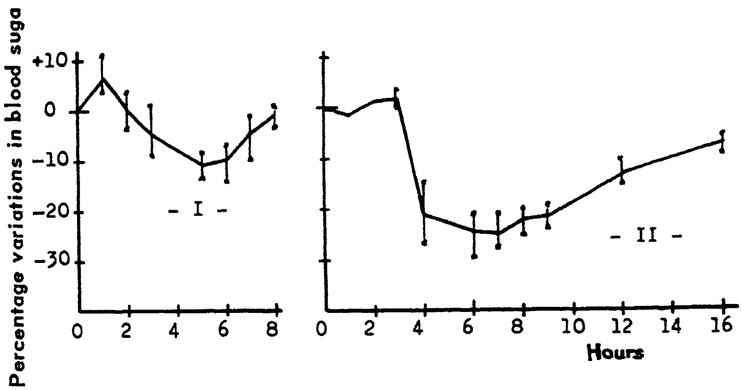


FIGURE 5. Effect on the blood sugar of the depancreatized dog of orally administered insulin, 10 units kg. (curve I), and of oral insulin given 3 hours after ingestion (time 0) of 2254 RP, 0.25 gm./kg. (curve II). Each curve shows the mean value and the range.

seems probable that the liver plays an important role in the phenomenon of potentiation.

It may be that enzymes such as insulinase,[12, 13] located in the liver, which normally destroy insulin, are inhibited by the sulfonamides, and that this forms part of the mechanism of potentiation; but this factor is certainly not the only one. In our experiments, high doses of sulfonamides were utilized (0.25 gm. per kg.). One may question whether, when therapeutic concentrations such as those employed in humans are used, this anti-insulinase activity intervenes at all or whether its role is very important. The sulfonylureas do not change the rate of degradation of labeled insulin *in vivo*,[45] and substances that inhibit insulinase are not always hypoglycemic. Moreover, it has been shown that the sulfonamides did not seem to inhibit a purified insulinase preparation. It appears to us that, if such a potentiation exists, it may exercise its effect following the liberation of a small quantity of endogenous insulin from the islets. The two mechanisms explain both the rapidity of the fall of blood sugar and also its duration.

Let us now consider the role of the liver in the hypoglycemic action of the sulfonamides. Although the role of the liver in the mechanism of blood sugar regulation is fundamental, the details are not all clear. It is no wonder then that the mechanism of action of the sulfonamides on the liver is still confused.

One important point stands out. Hepatectomy does not interfere with the acute lowering of the blood sugar caused by the sulfonamides, provided the pancreas is intact. This demonstration favors a pancreatic and extrahepatic action of these substances. This does not mean that the sulfonamides have no action on the liver, since the insulin that they liberate, or indirect modifications produced by it, may exert some action on that organ. Moreover, one cannot exclude the possibility of some hepatic influence during prolonged administration of a sulfonamide.

The drugs do not exert evident toxic actions in the normal animal, but it is possible that in pancreatic deficiency such a toxicity becomes overt, especially after prolonged administration. This has occurred in some experiments.[8] One can assert that these sulfonamides appear to be less toxic than synthalin. We may be dealing with some elective toxicity of one or another enzymatic system or cellular function.

The sulfonamides seem to favor formation of glycogen in the livers of normal animals.[1, 2, 3, 46, 47] This glycogen can be mobilized by epinephrine or by glucagon. We have always supposed that this glycogenogenesis was due to the liberation of small quantities of endogenous insulin into the portal system leading to the liver.

It has been demonstrated that glucose-6-phosphatase[36, 48, 49] and phosphorylase[36] could be inhibited *in vitro* by large concentrations of the sulfonamides. Does this occur *in vivo* and, if it does, is it sufficiently intense to explain the rapidity of the observed hypoglycemia? If that were true why does the totally depancreatized dog not show a decrease in blood sugar? Why does hypoglycemia not occur when these drugs are administered to severely alloxan diabetic animals or in very severe diabetes in man? The liver is present under all such conditions and contains the enzymes that should be inhibited; on the other hand, the number and functional capacity of the β cells are reduced as well. Finally, we are not sure that these enzymatic inhibitions are specific for the hypoglycemic sulfonamides.

Some endocrine glands or their products are able to modify the hypoglycemic action. We have shown that the administration of one of the sulfonamides does not interfere with the diabetogenic action of whole pituitary extracts[2, 3] and that the sulfonamide effect itself is modified so that an initial rise in blood sugar precedes a small fall.[15, 16] It would be interesting to do these experiments with purified growth hormone. One may ask if the diabetogenic extract has not modified the reactivity of the islet cells. Epinephrine hyperglycemia is not inhibited by the sulfonamides.[26, 36, 37] It is quite possible that the convulsive response shown by animals treated with these drugs depends upon an extra secretion of epinephrine and adrenocortical hormones.[12] It is also possible that the sulfonamides themselves depress nervous centers that are activated in the hypoglycemic state.[21]

We do not think that the action of the sulfonamides on thyroid function has any relation to the hypoglycemia, since thyroidectomy does not interfere with it.[10, 15] There are no demonstrable actions on the parathyroids nor on the gonads.[10, 15]

The effect of these drugs on the peripheral utilization of glucose has been studied in various ways. In isolated muscle[9a, 9b] and in the eviscerated animal[6, 9a, 9b, 46] the drugs do not increase glucose utilization. They do not elevate the R. Q. of the totally depancreatized animal.[2, 3] If an insulinlike action is manifested in the normal animal, it is probably insulin itself that is implicated. It should be remembered that contradictory results have been obtained in relation to glucose utilization.[50-53] These contradictory data have been obtained using various criteria, such as glucose tolerance, arteriovenous glucose differences, levels of intermediates in the blood, or glucose assimilation constants. The positive results are of value, since they have demonstrated that, if conditions are correct, the phenomena can be obtained.

In all such experiments we are testing the effects of endogenous insulin, and we do not know its exact physiological actions. It is possible, for instance, that what we call endogenous insulin is a mixture of varying proportions of insulin and glucagon. The exogenous insulin, which we know better, may only approximate the physiological secretion.

Analysis of the Antidiabetic Action of the Sulfonamides in Animals

By "antidiabetic action," we understand the following type of phenomenon. If one treats a moderately alloxan diabetic rabbit for several days with large doses of 2254 RP, one hastens the disappearance of the hyperglycemia and the glycosuria; at the same time its weight, which has been diminishing, stabilizes. The diabetes is ameliorated, and considerable metabolic improvement becomes manifest.[3, 5, 24, 28, 34] In some cases a potential diabetes remains and becomes overt when carbohydrates in larger amounts are administered. In other cases the diabetes disappears completely. The same phenomenon can be observed in the dog, but is much

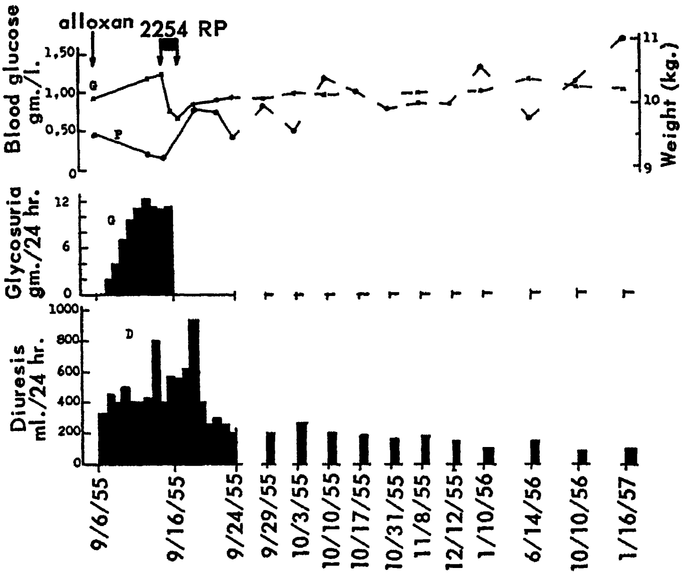


FIGURE 6. Alloxan diabetes in the dog "cured" by large doses of 2254 RP (2.5 gm./kg. over 36 hours, divided into 4 doses). P = body weight in lb.; G = fasting blood sugar level or glycosuria 24 hr.; D = urine volume, 24 hr.

more difficult to achieve (FIGURE 6). The intimate mechanism of this amelioration or disappearance of the diabetes is under study. In the pancreas of animals "cured" of their diabetes we have seen new formation of β cells.[5, 20, 24, 54] The most active substance in this respect is 2259 RP.

Since it has been shown already that the pancreas is necessary for the action of these drugs, and since the gland is the seat of a lesion provoked by alloxan, it is reasonable to conclude that the improvement is due to an intrapancreatic action. Experimentally, a very small degree of insulin deficiency is sufficient to elicit manifestations of the diabetic state; inversely, when the diabetes is mild, a small increase of insulin secretion will permit the alleviation of the diabetic state. It is therefore reasonable to suppose that the new formation of β cells augments the quantity of secreted insulin, thereby explaining the antidiabetic action observed. In some cases the effect is temporary, and the diabetic manifestations reappear progressively. It is also well established that if the alloxan diabetes is severe, no remission or improvement is possible.

Conclusions from Animal Experiments

We believe that acute administration of the sulfonamides acts primarily on the β cells to cause liberation of endogenous insulin. This insulin is secreted into the portal vein and reaches the liver cells directly. Large doses of the sulfonamides potentiate the effects of exogenous insulin. This phenomenon may be due to an anti-insulinase action in the liver, but this has not yet been specifically demonstrated. The chronic administration of the sulfonamides can cause a new formation of β cells, as well as a liberation of insulin from them. The metabolic benefit produced by these substances depends on the number of β cells that are not damaged or can be regenerated.

The action of these drugs on enzymatic mechanisms in the liver concerned with insulin destruction or with sugar metabolism is not yet completely explained. These enzymatic inhibitions are manifest only after large doses of the drugs, and they may or may not be specific. A stimulatory action of the sulfonamides on the adrenal glands may explain in some fashion the responses shown by treated animals to hypoglycemic convulsions.

Hypoglycemic Action of the Sulfonamides in Human Diabetics

In normal humans and in some diabetic patients the hypoglycemic action of the sulfonamides is easily observed.[25, 31, 33, 56, 57] The hypoglycemia may require intravenous injections of glucose.[56-58] Its manifestations are often insidious; we have compared them to those seen after large doses of depot insulin. If the low blood sugar persists for several hours in a fasting individual, central nervous system manifestations occur. The degree and the rapidity of the hypoglycemia obtained by a test dose of one of the drugs seem to be in accord with our knowledge of the insulin contained in the normal and diabetic pancreas.

In the depancreatized human the sulfonamides do not lower the blood sugar.[51, 59-61] On the other hand, in a hypophysectomized human who shows adrenal and thyroid deficiency, sulfonamide hypoglycemia can be produced.[62]

These drugs depress thyroid function temporarily,[63, 64] but even on continued treatment this hypothyroid condition does not persist in most cases. The excretion of 17-ketosteroids and of 17-hydroxycorticoids is not changed. The effects of epinephrine and of glucagon are not modified.[26, 59, 65, 66] The sulfonamides do not appear to exhibit much toxicity in the liver in the doses utilized clinically, since various tests of hepatic function are in general not depressed. It is important to reserve judgment about effects on the liver after very prolonged use of the drugs.

Contradictory results have been obtained concerning the effects of the drugs on glucose tolerance. Some patients respond rapidly and in a favorable manner, others only after many weeks.[52]

A potentiating effect on exogenous insulin has been observed in the diabetic, but the phenomenon is not constant. The response may depend on the type of diabetes and on the manner in which insulin and the drugs have been used. It is interesting to record that in some insulin-resistant patients the administration of sulfonamides has sometimes broken the resistance.

Antidiabetic Action of the Sulfonamides Observed in the Human

The antidiabetic action observed in human diabetics consists of a temporary remission or an apparent cure of the diabetes for several weeks after the cessation of treatment, with the restoration of a normal glucose balance. This phenomenon is relatively frequent in diabetics over 50 years of age, but it has been seen in younger individuals, particularly those with diabetes of recent onset. We have seen a girl of 15 whose parents and grandparents died of diabetic complications, including coma, in whom 2254 RP given for 15 days seemed to cause a regression of diabetic manifestations for 7 months (unpublished). Other authors have published similar observations.[67-69] It is true that, scientifically speaking, we should interpret such phenomena with reserve, but it does appear that, in such cases, the remission was due to β-cell regeneration which permits the individual to enter a phase similar to the prediabetic state. It would be difficult to interpret these results as due to reversible chronic liver depression. The reappearance of diabetes after remission could be explained by extrapancreatic factors (pituitary, adrenals, thyroid) or intrapancreatic conditions (α cells?) which break down the provisional and precarious restoration of the glucose balance.

We have made clinical trials using thiodiazole derivatives and the sulfonylureas in 160 patients.[21, 55-57, 70, 71] The thiodiazoles 2254 RP and 2256 RP appear to be less active than either carbutamide or tolbutamide, but 2259 RP (the tertiary butyl derivative) is at least as active and well tolerated as any of the urea compounds.

We have divided our patients into three groups on the basis of their response to sulfonamides:

The first group comprises the patients who have reacted favorably to the sulfonamide regimen. This group consists in general of patients of the lipoplethoric type over 45 years of age.[72] These patients tolerate the diabetic state well, but need insulin because of glycosuria and complications. In this group the sulfonamides were generally able to replace 20 to 30 units of insulin (sometimes up to 70 units). The diabetic state was ameliorated, and trophic, cutaneous, and vascular complications were temporarily halted. After cessation of sulfonamide therapy, the complications reappeared at the same time as did the glycosuria. A second therapeutic trial led to another remission (FIGURE 7). It was possible to do surgery in diabetics on sulfonamide therapy alone. The diet was restricted to 150 gm. of carbohydrate per day.

It should be noted that this first group of patients corresponds clinically to those with a pancreas containing many β cells and an insulin content about 50 per cent of normal.[73, 74] In such patients, 2 hours after glucose the blood contains insulin at a level of 70 per cent of normal.[75] All such individuals have a local deficiency of insulin in their pancreases, but their intracellular insulin appears to be difficult to mobilize. This phenomenon could be interpreted as a state of paresis of the β cells.

One may ask whether the amelioration of some of the complications may not depend in part upon the bacteriostatic action of the sulfonamides.

The second clinical group consists of patients who have not benefited from therapy. These are mainly young people, but some older diabetics are also included. Insulin treatment was obligatory because ketosis had appeared when the hormone was withdrawn. These patients belong to the group completely deficient in insulin.[72] The pancreases of such patients have few intact

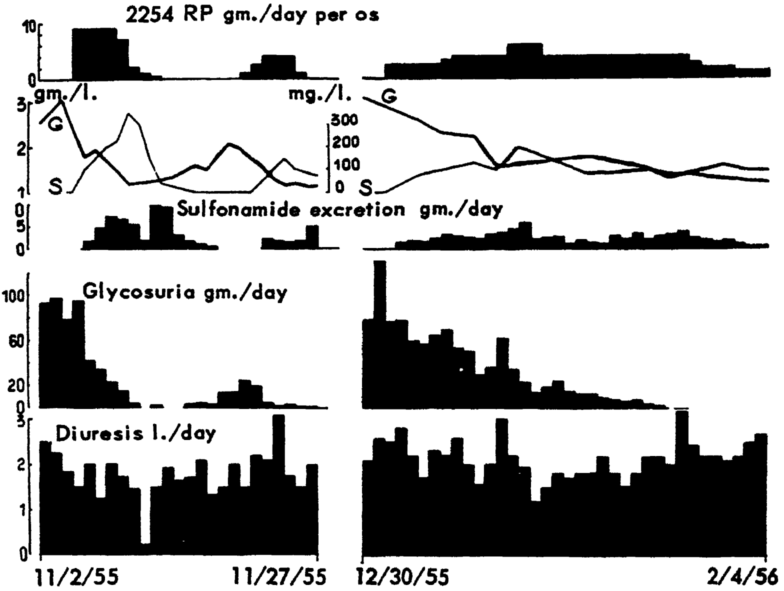


FIGURE 7. Diabetic patient, Mrs. M. Ch., 66 years old. Insulin therapy had been required for 3 years. Insulin withdrawal led to intense diabetes. The left portion of the graph shows the first period of sulfonamide therapy. The right side shows a second period of the same therapy. Between these periods the patient received neither insulin nor sulfonamide for 1 month. Ulcerations on the plantar portions of her feet disappeared when sulfonamides were given, reappeared during the interval between treatment periods, and disappeared again during the second treatment. Her general health was excellent under this therapy, and her weight increased. G = fasting blood sugar; S = blood sulfonamide.

β cells, and the insulin content is close to 10 per cent of normal.[73, 74] The blood of such patients contains little, if any, detectable insulin. In some cases in this group the sulfonamides actually intensify the diabetic state. It is true that in some young patients with diabetes of recent onset sulfonamide treatment has produced remissions, but these are rare instances.

The third group comprises those patients who have benefited partially. It was possible to reduce but not completely to eliminate insulin. This partial benefit may be due to some liberation of endogenous insulin, to the potentiation effect, or perhaps to new formation of cellular elements in the pancreas.

One sees, therefore, that in man as well as in animals the functional apparatus of the pancreas is linked intimately to the action of the sulfonamides. The β cells and their hormonal product play an important role in this action. This mechanism does not exclude other modes of action, but we believe it to be the most important. We shall accept other mechanisms when they are demonstrated. It is interesting to note that investigators of authority in the field, independently and also simultaneously with us, have seen that the efficacy of the sulfonamide treatment depends upon the type of diabetes (growth-onset or maturity-onset).[71, 73] The validity of the theory which we have proposed and maintained can explain the state of our knowledge at present, even though some of the effects are left unclear. This is for the future to unravel. Certain reservations should be mentioned now.

(1) When one uses the sulfonamides for the chronic therapy of diabetes mellitus, one must bear in mind the possibility that a decompensation and weakening of the β-cell system may occur. With judicious administration and good clinical indications, one would expect this to be a rare occurrence.

(2) As a chemical group, the hypoglycemic sulfonamides are probably not devoid of toxicity. They may provoke sensitization; they may accumulate in various organs or tissues; and some enzymatic systems may be blocked in the course of long-term administration. It is also possible that some individuals originally sensitive to their action may later become refractory. Such cases are extremely rare.

The drugs under discussion are of interest for the study and for the possible treatment of diabetes, and some of the results obtained in man are highly impressive. There is reason to feel that further research may develop a substance having the essential property of the sulfonamides, but without certain of their inconveniences. We believe that these drugs have already been the occasion of much valuable research in the physiology, pharmacology, and pathogenesis of diabetes. One must reserve complete judgment in relation to treatment.

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METABOLIC EFFECTS OF SULFONYLUREAS IN NORMAL MEN AND IN VARIOUS TYPES OF DIABETIC PATIENTS*

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The studies described in this report were initiated in an effort to elucidate the mechanism of action of the sulfonylurea compounds. It seemed to us that a broad clinical investigative approach, involving the study of a large number of conditions in which the metabolism of carbohydrate is disturbed, was most likely to eliminate quickly a number of possible modes of action and to define more sharply the areas upon which further effort should be concentrated. Thus, extensive metabolic-balance studies and numerous individual testing procedures have been performed before, during, and following the administration of carbutamide (BZ-55) and/or tolbutamide (Orinase§) in the following subjects: (1) healthy young men; (2) three middle-aged, obese, stable diabetics; (3) an unstable diabetic of normal weight; (4) a patient with lipo-atrophic diabetes; (5) a totally depancreatized woman; (6) patients with coexisting diabetes mellitus and Addison's disease, familial diabetes and Cushing's syndrome, and diabetes and panhypopituitarism; and (7) an acromegalic with mild diabetes.

Although the data reported below do not define a specific mode of action of the sulfonylurea compounds, they eliminate from consideration a number of important possibilities.

Results

Administration of the sulfonylurea compounds to normal men produces definite hypoglycemia. A single dose of either 3 or 6 gm. of tolbutamide given to 2 normal subjects in the fasting state produced decreases in blood sugar of 35 to 60 per cent.

In normal subject D. M., administration of 6 gm. of carbutamide as a single dose on 2 consecutive days, followed by a dose of 3½ and 4 gm. daily in divided doses for the next 2 days, produced a fall in the level of fasting blood sugar from 78 to 24 mg. per cent (FIGURE 1). Subsequent administration of 3 gm. daily in divided doses kept levels of fasting blood sugars fairly constant at about 60 mg. per cent.

In the same normal subjects, when the compounds were administered at

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§ The Upjohn Company, Kalamazoo, Mich.

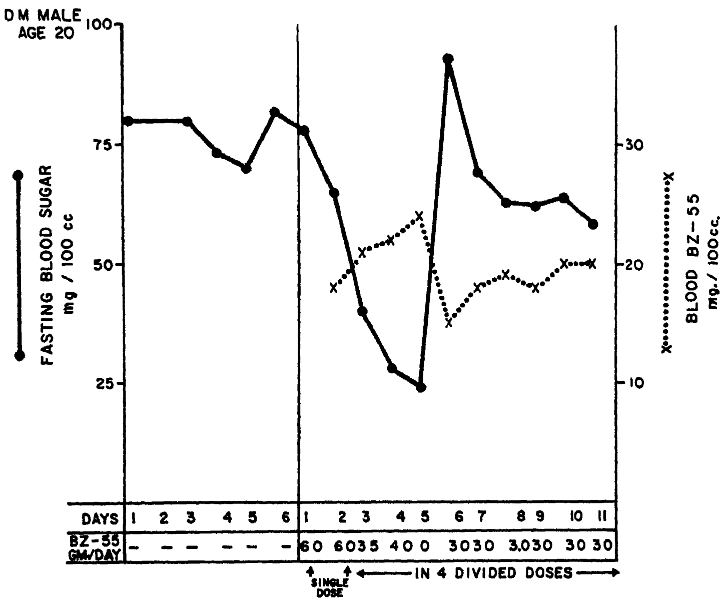


FIGURE 1. Effect of the administration of carbutamide (BZ-55) on fasting blood sugar in a normal subject.

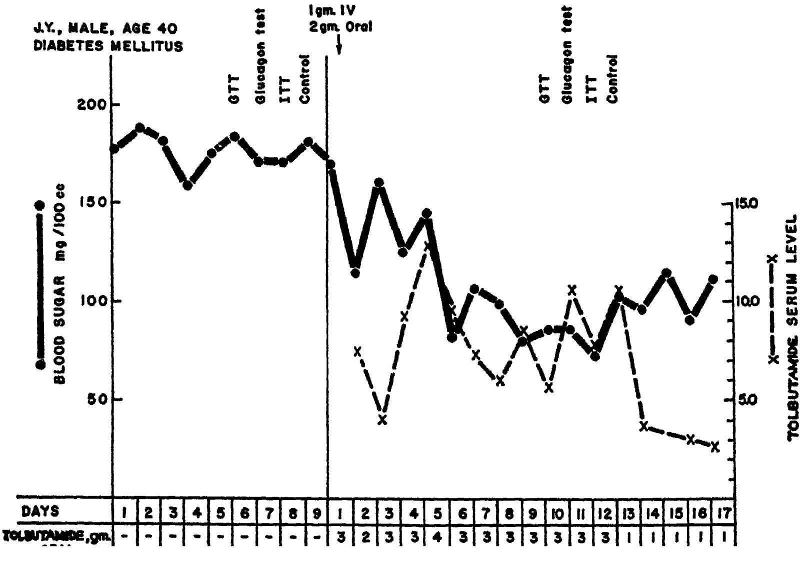


FIGURE 2. Effect of the administration of tolbutamide on fasting blood sugar in a patient with diabetes mellitus.

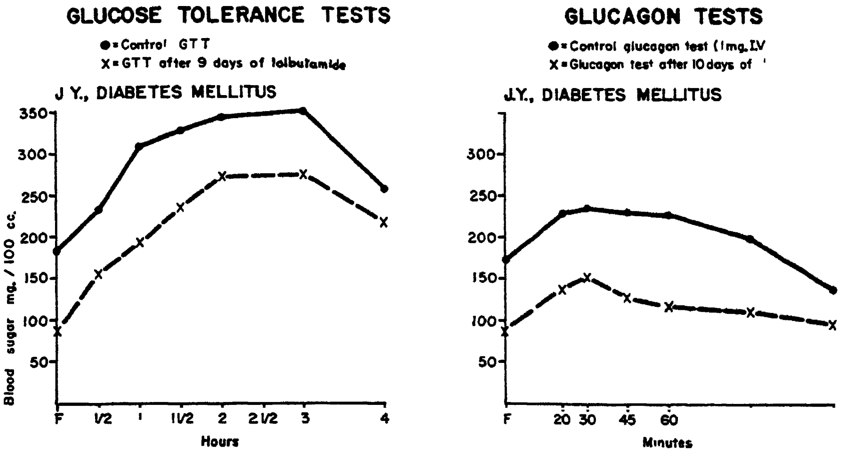


FIGURE 3. Effect of tolbutamide on glucose tolerance and hyperglycemia produced by glucagon in a diabetic patient.

the same level of dosage, tolbutamide was less effective than carbutamide in reducing the daily fasting blood sugar.[1]

FIGURE 2 demonstrates that administration of tolbutamide to a mild diabetic produced a decrease in levels of fasting blood sugar from the hyperglycemic into the normal range.

FIGURE 3 shows that glucose tolerance was not altered in this patient by the administration of tolbutamide for 9 days, even though the fasting blood sugar was then in the normal range. Glucose tolerance was also not influenced significantly during the administration of sulfonylurea compounds to healthy individuals.[1]

In FIGURE 4 we see that intramuscular administration of adrenalin produced the same rise in blood sugar in a normal male, whether given before administration of carbutamide or after the drug had effected a lowering of the blood sugar in the fasting state. Similarly, the hyperglycemic response to intravenously administered glucagon was not blocked by administration of these drugs in normal subjects,[1] or in the diabetic patient (FIGURE 3).

The sulfonylurea drugs did not potentiate the activity of exogenous insulin in normal males or in diabetic subjects.

FIGURE 5 shows the results of insulin tolerance tests performed in a normal male. The solid line represents the control insulin tolerance test. The line connecting the open circles shows the blood sugar curve obtained during the carbutamide period, but without administration of insulin. Administered insulin at this time was no more effective than it had been before carbutamide had produced a hypoglycemic effect, as shown by the line connecting the crosses.

FIGURE 6 shows similar data obtained in a diabetic. The middle curve represents the results of a standard insulin tolerance test. The lower 2 curves depict the results of insulin tolerance tests performed after administration of

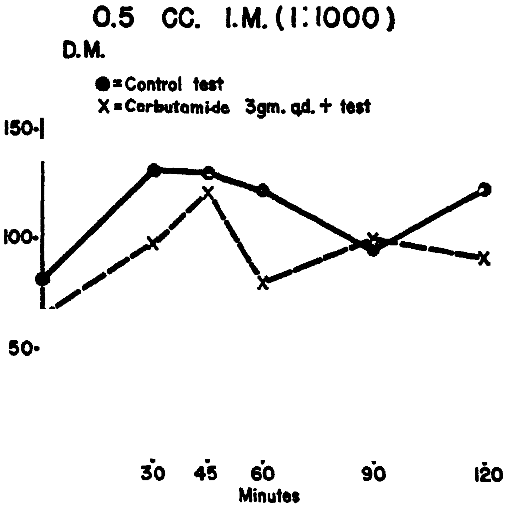


FIGURE 4. Effect of carbutamide on hyperglycemia produced by adrenalin in a norma subject.

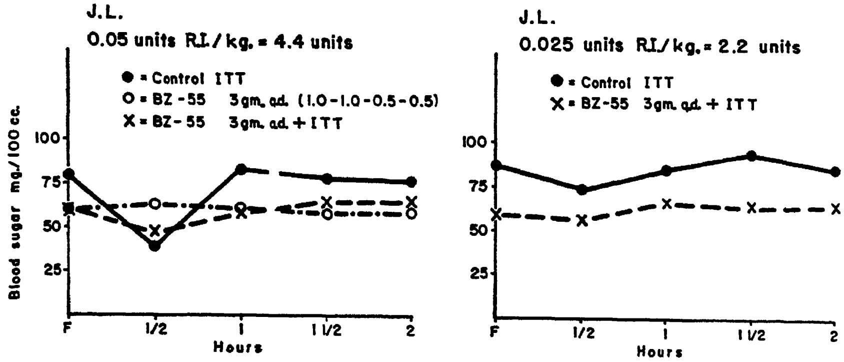


FIGURE 5. Effect of carbutamide (BZ-55) on an insulin tolerance test in a normal subject.

tolbutamide for 4 or 5 days, respectively. Again, no potentiation of insulin activity is demonstrated.

In addition, in 3 of our diabetic patients in whom the drugs did not produce a lowering of blood sugar, there was no difference in insulin requirement whether the diabetes was controlled with insulin plus sulfonylurea or with insulin alone. One of these 3 patients is the boy with panhypopituitarism and diabetes, who is very sensitive to insulin and in whom any potentiation of insulin activity should have been very easily observed.

That decreased function of the pituitary-adrenal system is not the mode of action of these compounds is indicated by the following observations:

(1) No significant changes in renal excretion of 17-hydroxycorticoids or

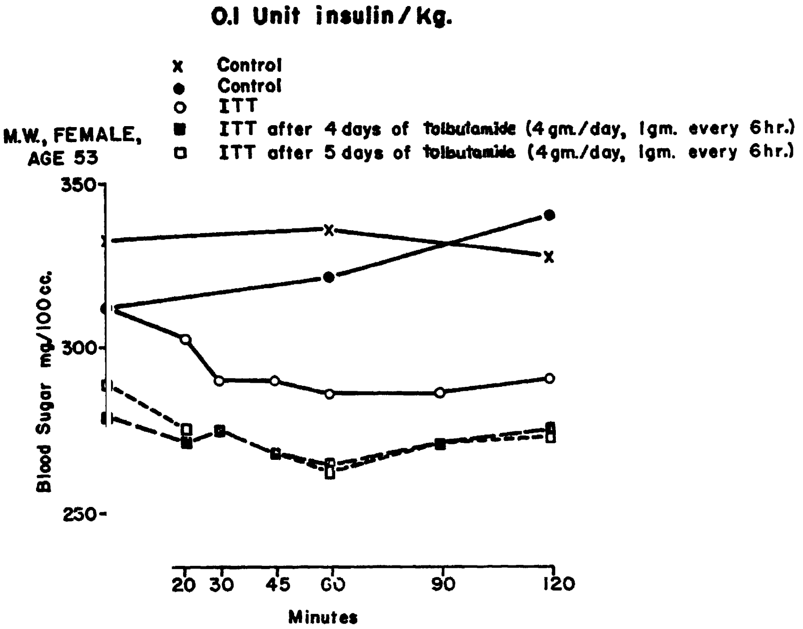


FIGURE 6. Effect of tolbutamide on an insulin tolerance test in a diabetic subject.

17-ketosteroids could be demonstrated in healthy men or in diabetic patients during the administration of the drugs.[1]

(2) There were no significant changes in the balances of nitrogen, sodium, chloride, or potassium on administration of the sulfonylureas.[1]

(3) In the patient with coexisting diabetes mellitus and Addison's disease, administration of tolbutamide reduced the level of fasting blood sugar from about 150 to 110 mg. per cent, despite constant replacement therapy with hydrocortisone and 9α-fluorohydrocortisone. Withdrawal of the steroids for 2 days did not potentiate the hypoglycemic effect of tolbutamide in spite of the development of severe adrenal insufficiency.[1]

(4) In 5 nondiabetic patients with severe adrenal insufficiency, intravenously administered sodium tolbutamide produced a similar hypoglycemic effect whether the patients were maintained on hydrocortisone and 9α-fluorohydrocortisone or with deoxycorticosterone alone.

As shown in FIGURE 7, the sulfonylurea compounds do not act by blocking the peripheral effects of adrenocortical steroids. At a time when carbutamide had produced a definite hypoglycemic effect, the administration of 100 mg. of Prednisolone for 2 days produced the same rise in fasting blood sugar and the same loss of carbohydrate tolerance as when Prednisolone was given by itself. The sulfonylureas did not alter any of the metabolic changes produced by Prednisolone.[1]

The sulfonylurea compounds are not substitutes for insulin. Carbutamide had no effect in the pancreatectomized patient. In the unstable diabetic,

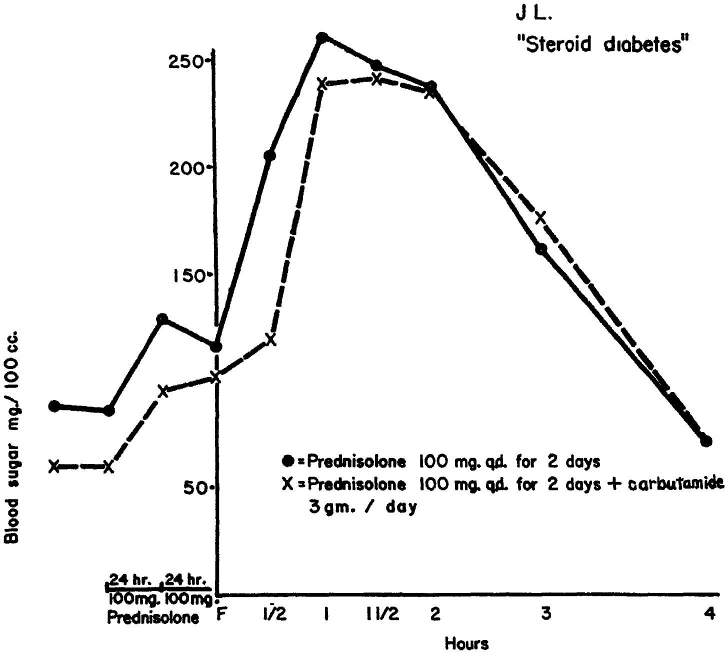


FIGURE 7. Effect of Prednisolone on fasting blood sugar and glucose tolerance before and during administration of carbutamide.

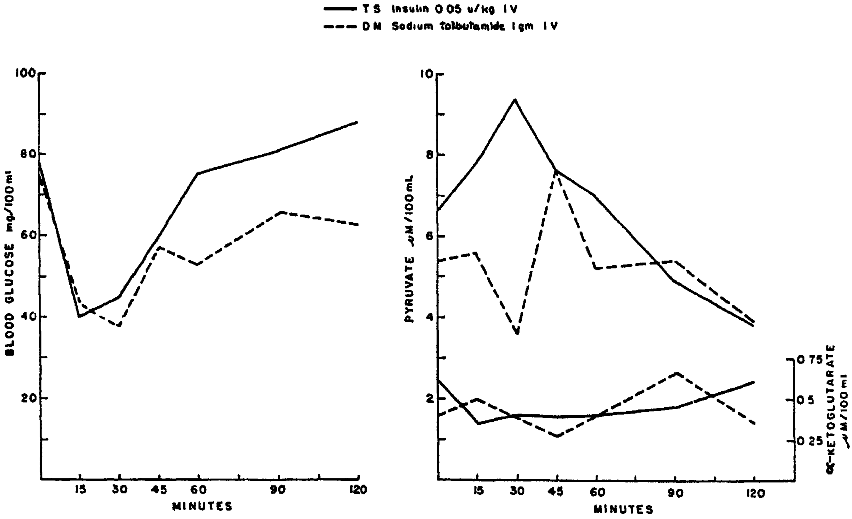
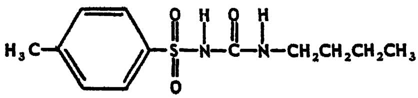


FIGURE 8. Effects of intravenous administration of insulin and of sodium tolbutamide on the blood levels of glucose, pyruvate, and α-ketoglutarate.

Tolbutamide



Tolbutamide excretion product

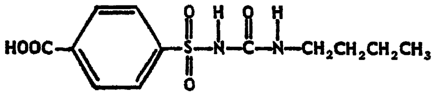


FIGURE 9

withdrawal of insulin and administration of either carbutamide or tolbutamide resulted in hyperglycemia, glycosuria (which at times equalled the intake of carbohydrate), ketonuria, and negative balances for nitrogen, sodium, and potassium.[1]

FIGURE 8 shows an example of the findings obtained during 8 intravenous insulin tolerance tests and 7 intravenous tolbutamide tests performed in normal subjects. Hypoglycemia induced by intravenous administration of insulin was associated with an initial rise in blood pyruvate. In contrast, when hypoglycemia was induced by intravenous administration of sodium tolbutamide the earliest change in levels of blood pyruvate was a decrease.[2] These differences suggest that the immediate hypoglycemia induced by administration of insulin on the one hand, and of tolbutamide on the other, occurs via different mechanisms.[2]

During oral administration of tolbutamide it was noted that, upon acidification of the urine to *p*H 5.2 or below, a precipitate appeared. At *p*H 3 precipitation was fairly complete. Subsequently, a crystalline compound was isolated with a melting point different from that of tolbutamide. In cooperation with Struck, Wright, and Johnson of The Upjohn Laboratories, this urinary excretion product of tolbutamide has been identified as a carboxylic acid derived by oxidation of the methyl group linked to the aromatic ring (FIGURE 9).[3]

When tolbutamide was administered to normal subjects at a level of 3.0 to 6.0 gm./day, 70 to 80 per cent was excreted in the urine as this excretory product. When 3 to 4 gm./day was administered orally to diabetic subjects, excretion as the metabolite ranged between 24 and 67 per cent. This material is very likely the principal excretion product of tolbutamide in the human.[1]

Intravenous administration of the pure tolbutamide excretion product as the sodium salt to 4 normal subjects and 1 diabetic patient in a dosage of 1 to 3 gm. failed to elicit any blood-sugar-lowering effect. Oral administration of 6 gm. of the excretion product daily for 3 days in 4 divided doses to a normal subject likewise failed to influence the level of fasting blood sugar.[1]

Summary and Conclusion

The data indicate that the sulfonylurea compounds do not (1) suppress the pituitary-adrenal system, (2) antagonize the peripheral effects of adrenal corticoids, (3) block the hyperglycemic effects of glucagon and adrenalin, (4) potentiate the activity of exogenous insulin, and that (5) they are not insulin substitutes. Thus, the blood-sugar-lowering property of these compounds is achieved via another mechanism.

The contrasting initial changes in blood pyruvate level associated with hypoglycemia induced by insulin and tolbutamide, respectively, suggest that the sulfonylureas produce *acute* hypoglycemia by a mechanism other than the rapid release of endogenous insulin.

When tolbutamide is administered, a carboxylic acid derivative of this compound is excreted in urine as a major excretory product.

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EFFECTS OF TOLBUTAMIDE ON THE DIABETES OF ACROMEGALY AND ON THE BLOOD SUGAR IN PATIENTS WITH ALTERED ENDOCRINE STATES

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Despite the recent numerous reports demonstrating the hypoglycemic effect of the arylsulfonylurea compounds, the mechanism of their action remains unknown. The experiments reported indicate numerous sites of action; for example, the compounds have been reported to show an increase in the secretion of insulin by the pancreas;[1] decrease in glucagon release, with destruction of α cells of the pancreas;[2] increase in anti-insulinase activity;[3] and decrease in glycogenolysis, with decrease in the release of glucose from the liver to the blood stream.[4]

The studies presented in this report were undertaken to determine whether the action of tolbutamide (Orinase*) was dependent upon alterations in the secretion of endocrine glands other than the pancreas and to ascertain whether the alteration of the body's endocrine balance would give information as to the mechanism of response to tolbutamide.

The possible role of growth hormone as a factor in the production of diabetes mellitus in man is as yet unknown. In certain species of animals the administration of bovine growth hormone will produce severe diabetes mellitus. The clinical syndrome of acromegaly, which is characterized by an abnormal increase in the growth of many body structures, frequently is associated with diabetes mellitus.[5] The factors responsible for the diabetes have not as yet been clearly delineated. The diabetes has been reported to be insulin-resistant, and at least one investigator has found increases in circulating insulin in the blood of such patients.[6] Increased function of the adrenal cortex has been implicated by other investigators.[7] The growth hormone itself may have an anti-insulin action, either by peripheral inhibition or by stimulating the production of increased amounts of plasma bound inactive insulin that will, over a prolonged period of time, result in exhaustion of the β cells of the pancreas and permanent diabetes mellitus.

Attempts were also made to study the effect of tolbutamide in endocrine conditions that may result in a disturbed carbohydrate metabolism. Such alterations in the patient's endocrine balance as those caused by adrenalectomy, hypophysectomy, administration of excessive amounts of cortisone, administration of adrenocorticotropic hormone (ACTH), and hyperthyroidism were studied in relation to changes in carbohydrate metabolism following administration of tolbutamide.

Although the results of these studies do not permit a definition of the mode of action of tolbutamide, they provide observations that demonstrate clearly the fact that tolbutamide may exert its hypoglycemic effect in the absence of, or without change in the function of, certain endocrine glands. The degree of the hypoglycemic response following tolbutamide may be altered with changes in the levels of certain hormonal substances in the body.

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* The tolbutamide (1-butyl-3-*p*-tolylsulfonylurea) and amphenone (1,2-bis(*p*-aminophenyl)2-methyl-propanone-1) used were supplied through the courtesy of The Upjohn Company, Kalamazoo, Mich.

Methods

The general plan of this study was to investigate patients with acromegaly associated with diabetes mellitus who were on metabolic balance studies and other patients with altered endocrine states who were maintained on regular diet. Insulin therapy of the diabetic patients was withdrawn for the duration of the study, and the patients were placed on a constant diet. Twenty-four-hour urine specimens were collected for the determination of total nitrogen,[8] phosphorus,[9] calcium,[10] creatine, creatinine,[11] α-amino acid nitrogen,[12] glucose,[27] sodium, potassium, and chloride. Periodic determinations of blood[14] and urinary 17-hydroxycorticosteroids[15] and urinary 17-ketosteroids[16] were performed. Blood sugars[13] were obtained fasting and 2 hours postprandially.

The other patients studied were hospitalized and maintained on a regular diet The adrenalectomized and the hypophysectomized patients were maintained on cortisone. A group of special tests was performed during the course of these studies, and each patient had one or more of these special tests. The 5-hr. fasting test was performed by withholding breakfast and determining the blood sugars hourly for 5 hr. Arterial blood was obtained by finger stick in all tests. The fasting tolbutamide test was performed by withholding breakfast, giving a 2-gm. oral dose of tolbutamide, and drawing samples for blood sugars hourly for 5 hr. The insulin tolerance test was done by giving intravenously 0.05 or 0.1 units of crystalline insulin per kilogram of body weight. The oral glucose tolerance test was performed by giving 100 gm. of glucose orally and determining the blood sugars hourly for 5 hr.

The intravenous glucose tolerance and intravenous glucose-insulin tolerance tests were performed by the injection of 25 gm. of glucose intravenously over a 3-min. period; when insulin was also administered, it was given at a dose level of 0.05 to 0.1 units per kilogram of body weight at the end of the glucose injection. Blood sugars were determined at frequent intervals up to 60 min. after the end of the glucose injection. From these tests the percentage of glucose disappearance per minute can be determined. The normal rate of disappearance is 3 to 5 per cent per minute, and a moderate diabetic would show a disappearance rate of about 2 per cent per minute.[17]

RESULTS AND COMMENTS

Patients with Acromegaly and Diabetes Mellitus

The first patient, C. R., is a 65-year-old white female who 10 years ago noted gradual enlargement of her face, hands, feet. and tongue, which over the past 10 years have progressively increased in size. In the last 3 years the rate of growth has been less than in the early stages of her disease. She now has the full characteristic findings of acromegaly. Shortly after the onset of the acromegaly, she was found to have diabetes mellitus and was started on 60 units of protamine-zinc-insulin (PZI) before breakfast and 10 units at bed time. Her diabetes was fairly well controlled on this insulin program; however, she continued to have occasional positive reactions for glucose in her urine.

The patient was placed on a constant diet (calories, 1,281; protein, 74 gm.; fat, 61 gm.; carbohydrate, 109 gm.). Her insulin was withdrawn and she was permitted to come to equilibrium before administration of tolbutamide was begun. The patient was given 1 gm. tolbutamide orally twice a day. The course of her diabetes, as reflected by the changes in her blood and urine sugars, is shown in FIGURE 1. There was a prompt drop in the blood and urine sugar. It is interesting to note that, following discontinuation of tolbutamide, the blood sugars again rose, but not to the pretreatment levels. Following discharge from the hospital the patient has been maintained on 2 gm. tolbutamide a day, but has not followed the recommended diet carefully. She has done well, nevertheless, except for one period when a pulmonary infection resulted in exacerbation of her diabetes and necessitated insulin therapy for a period of one week. There has been no toxic manifestation during the administration of tolbutamide.

During the period of administration of tolbutamide there was a tendency to a more positive nitrogen balance, which became apparent near the middle of the course and persisted after discontinuation of the drug (FIGURE 2) This positive balance may indicate less catabolism of protein secondary to better glucose utilization. The phosphorus balance was significantly nega-

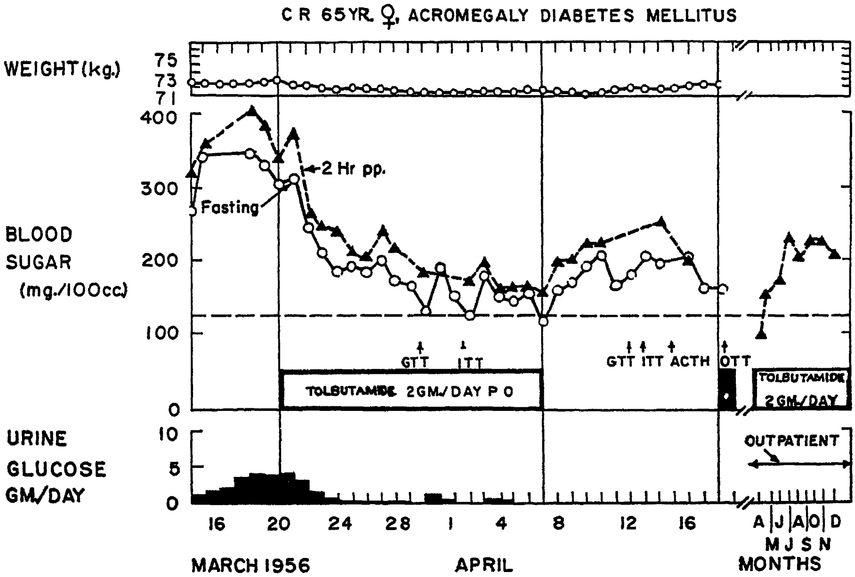

FIGURE 1

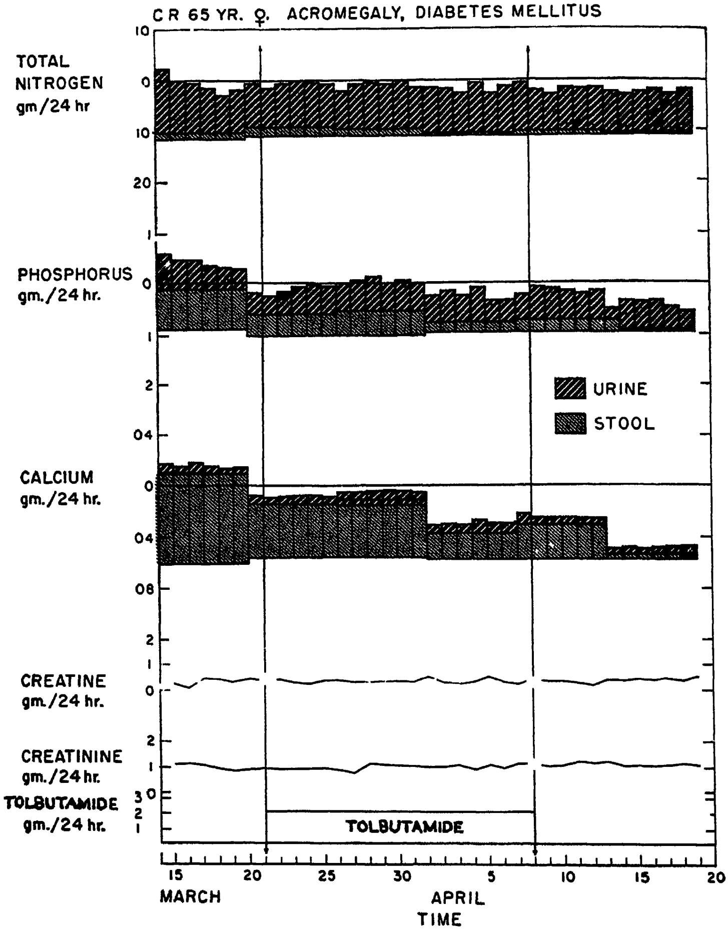

Figure 2

tive during the pretreatment period and became progressively positive as the diabetes was controlled. This may have resulted from less protein catabolism or from more effective storage of glucose in its phosphorylated form. The explanation of the increasing positive calcium balance is not clear at this time. The effect of tolbutamide on the electrolyte excretion is shown in FIGURE 3; there seems to be no significant alteration in excretion of sodium, potassium, or chloride.

There was observed no significant alteration of adrenal cortical function, as measured by blood and urinary 17-hydroxycorticosteroids and by urinary 17-ketosteroids, during tolbutamide administration (FIGURE 4). Thus, in this type of diabetes, the hypoglycemic effect does not seem to be mediated through depression of adrenal cortical function. No other significant change in blood constituents during the administration of tolbutamide was noted.

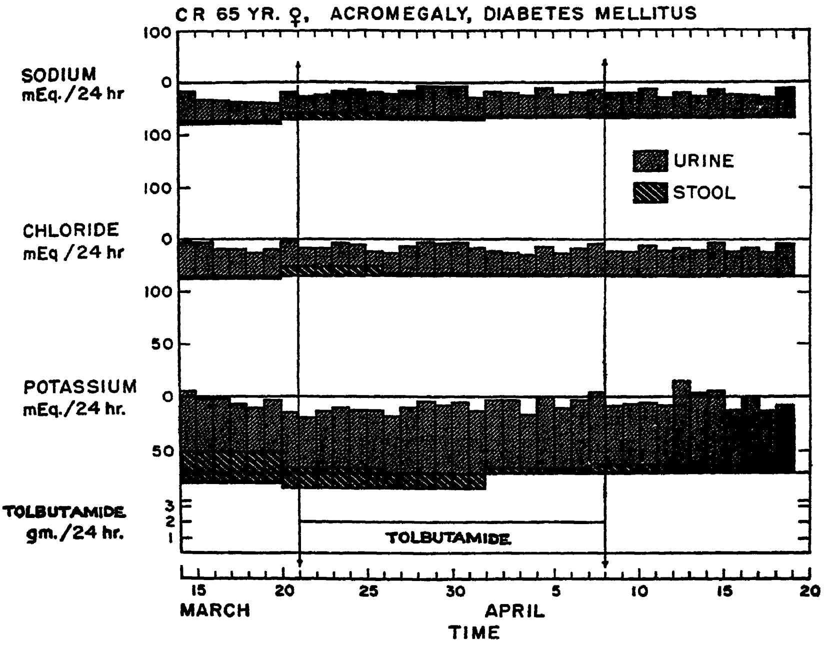

FIGURE 3

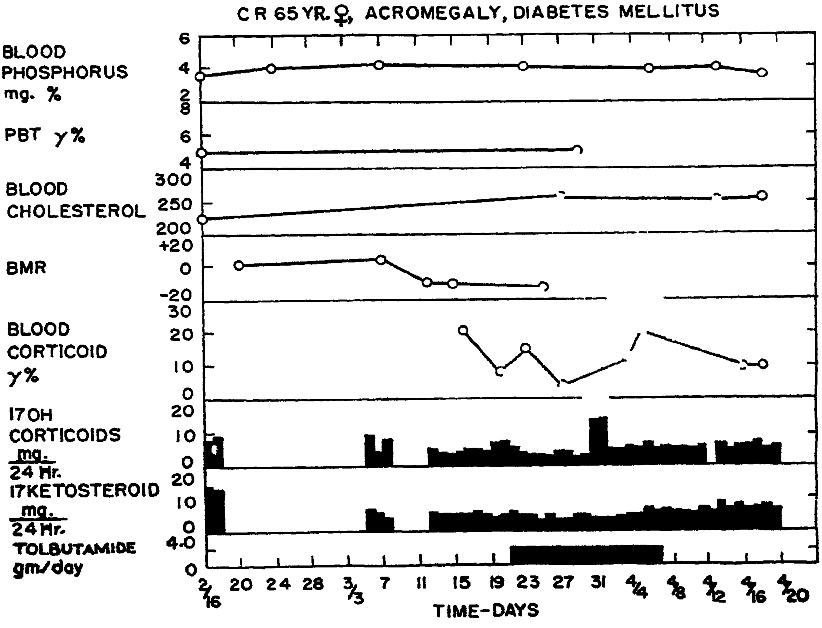

FIGURE 4

C.R. 65 YR. ♀, ACROMEGALY DIABETES MELLITUS

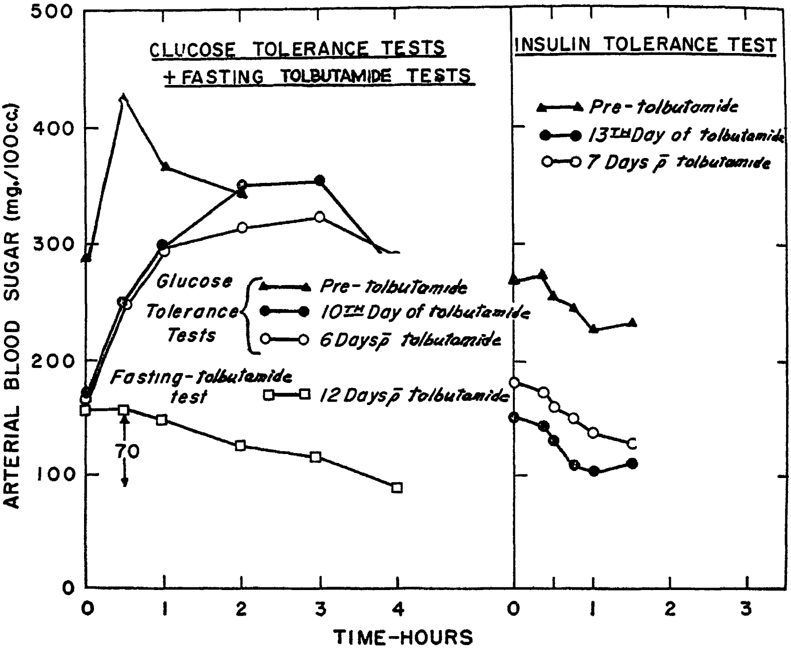

FIGURE 5

A fasting tolbutamide test demonstrated a decrease in the blood sugar of 70 mg. per cent at the end of 5 hr. This test was performed 12 days after the tolbutamide had been discontinued and the hyperglycemia had returned (FIGURE 5). The 3 oral glucose tolerance tests are shown in FIGURE 5. Before the tolbutamide therapy there was a severe hyperglycemic curve, and in 2 subsequent tests, performed on the tenth day of therapy and 6 days after the discontinuation of tolbutamide, the curves were found to be lower than the initial glucose tolerance curve and essentially equivalent to each other. All the curves are quite abnormal, indicating a typical diabetic type of response. The 3 insulin tolerance tests shown in FIGURE 5 reveal a relative insulin resistance that was not corrected by tolbutamide.

Intravenous glucose tolerance tests (GTT) were done at a time when the diabetes was untreated and again on the fifth day of tolbutamide therapy. The daily dose of tolbutamide was 3 gm., given as a single dose in the morning (FIGURE 6). A markedly diabetic curve was observed in a pretreatment test and following a single 2 gm. oral dose of tolbutamide given 3 hr. prior to the intravenous glucose. The rate of disappearance of excess glucose was 1 per cent per minute in both tests. When 7 units of crystalline insulin was given along with the glucose, the glucose disappearance rate increased to 2.5 per cent per minute, a value less than would be expected in a normally insulin-sensitive individual, but still indicating a significant increase in glucose dis-

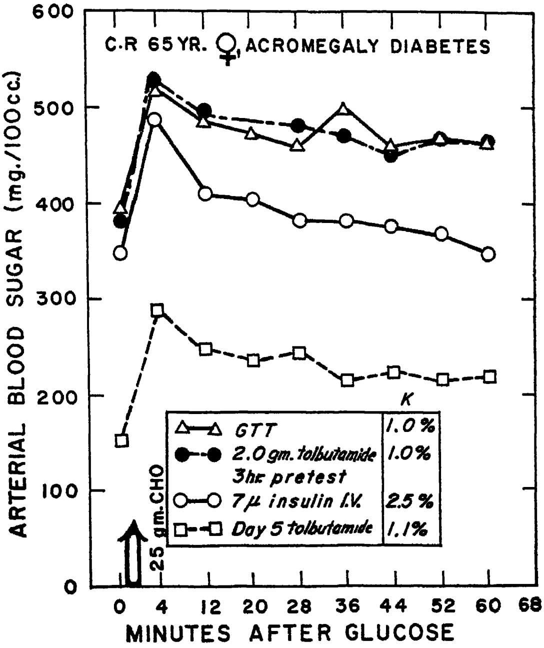

FIGURE 6 Glucose disappearance rates.

appearance over the control test. The test was repeated after the fifth day of tolbutamide therapy, when initial blood sugar values were significantly lower than during the pretreatment test, and the rate of glucose disappearance was found to be 1.1 per cent per minute, essentially the same as before therapy. Thus, although the blood sugars are significantly lowered by oral tolbutamide, the rate of disappearance of excess glucose after a glucose load is not handled in the same manner as when exogenous insulin is administered intravenously.

The second patient, O. W., is a 66-year old Negro male, who noted 15 years ago a gradual increase in the size of his hands, feet, and lower jaw, and migrating pains in the joints and in the muscles around the joints. A diagnosis of acromegaly was made, and an enlarged sella turcica was found. In 1953 the patient received X-ray therapy to the pituitary and felt that the increase of the hand and foot size had halted, although the arthritic symptoms have progressed. In 1952 the patient had symptoms of polydipsia and polyuria, and a diagnosis of diabetes mellitus was made. He was controlled with diet until the summer of 1954, when the hyperglycemia increased, and he was then given 15 units of NPH insulin daily. On his admission to the hospital, the insulin was discontinued and the patient was placed on a constant diet (calories, 2,602; protein, 123 gm.; fat, 114 gm.; carbohydrate, 271 gm.). During the period before balance studies were begun, he was given a number of glucose tolerance, fasting tolbutamide, and insulin tolerance tests.

Two courses of tolbutamide, 1 gm. twice daily, and one course of amphe-

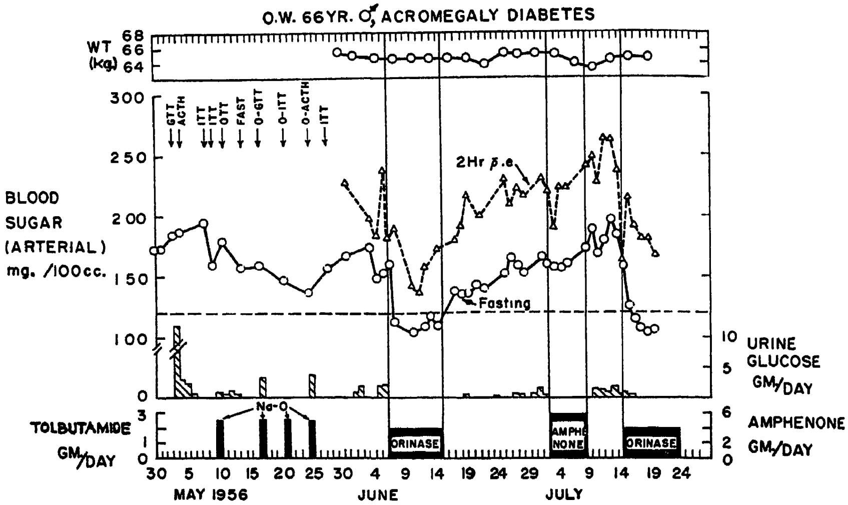

FIGURE 7

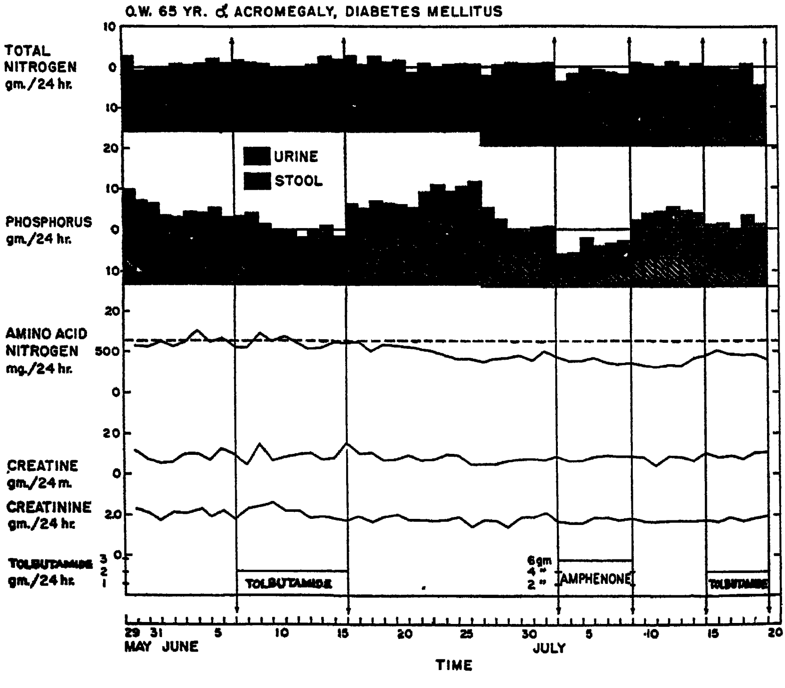

FIGURE 8

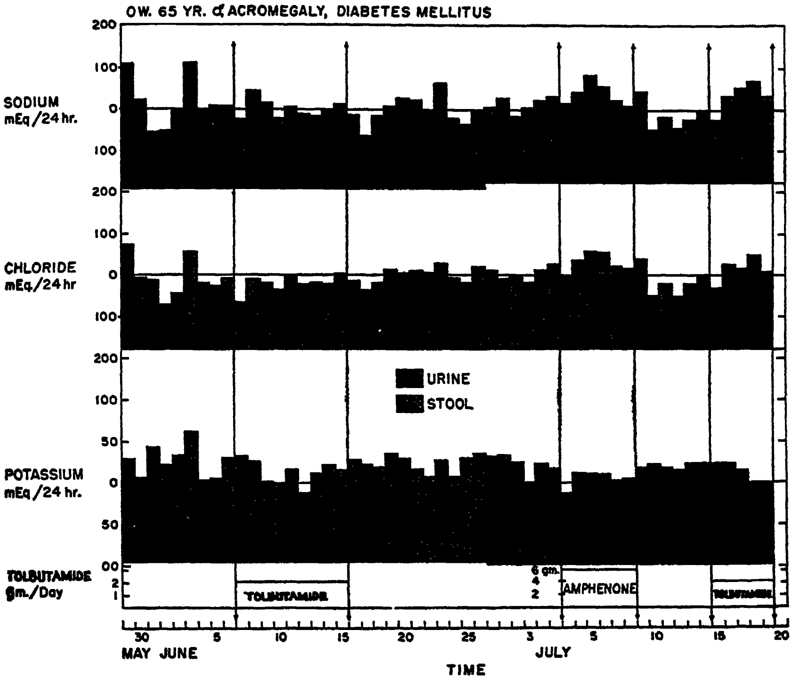

Figure 9

none, 1 gm. every 4 hr., were given to this patient. The results of these courses of therapy are shown in figure 7. There was a prompt decrease in the fasting and the 2-hr. postprandial blood sugars, which returned to a diabetic level when the tolbutamide was discontinued. The patient has now been adequately controlled for a period of 8 months on 2 gm. of tolbutamide a day. No toxic manifestations have been observed during administration of the drug.

To determine whether the adrenal cortex may play some role in the diabetes mellitus associated with acromegaly, the patient was given a course of oral amphenone. Amphenone has been shown by Hertz *et al.*[18] to be an effective agent in suppressing adrenal cortical function. At the dose level used, 6 gm. a day, there was evidence of slight but significant adrenal cortical suppression, as reflected in a drop in blood corticoids and a possible small decrease in urinary 17-hydroxycorticoids (figure 10). There did not appear to be any significant alteration in the course of the diabetes mellitus.

The effect of tolbutamide and of amphenone on the mineral balance of the patient is shown in figures 8 and 9. There was no significant alteration in nitrogen metabolism during tolbutamide administration, but a positive nitrogen balance was obtained during amphenone administration. A possible explanation for this could be a decrease in catabolism of protein secondary to a decrease in adrenocortical output. The α-amino acid nitrogen excretion is significantly elevated throughout the study. The elevated phosphorus

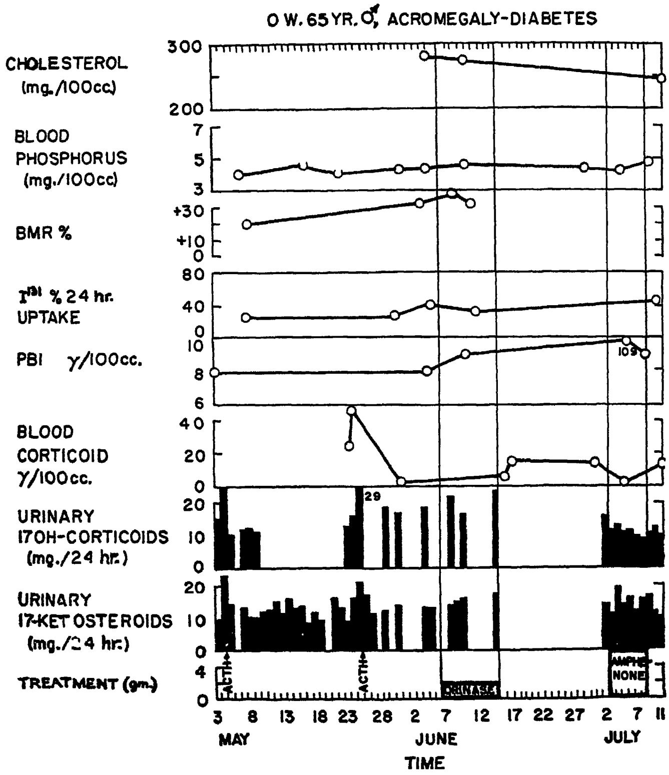

FIGURE 10

excretion following the first course of tolbutamide is most likely due to the administration of aluminum hydroxide, an antacid medication, which was given to the patient during the week following discontinuation of tolbutamide.

No significant changes in excretion of sodium, potassium, or chloride were noted during the tolbutamide administration, but there was evidence of a sodium diuresis and a slight potassium retention during amphenone administration, with subsequent sodium retention and potassium loss in the post-treatment period (FIGURE 9). Recently, Liddle *et al.*[19] and Renold *et al.*[20] have suggested that amphenone is capable of suppressing the secretion of aldosterone by the adrenal cortex, and the changes observed in the present experiment would be compatible with this suggestion.

There was no significant change in the excretion of urinary-17-hydroxy-corticosteroids or 17-ketosteroids during tolbutamide administration (FIGURE 10). Although the B.M.R. and protein-bound iodine (PBI) were somewhat elevated, the uptake of radioactive iodine by the thyroid gland was within normal limits. The patient did not have the clinical symptoms of hyperthyroidism.

A 5-hr. fast resulted in a 27 mg. per cent drop in blood sugar. When 2 gm. of oral tolbutamide was given at the beginning of the fasting period, the

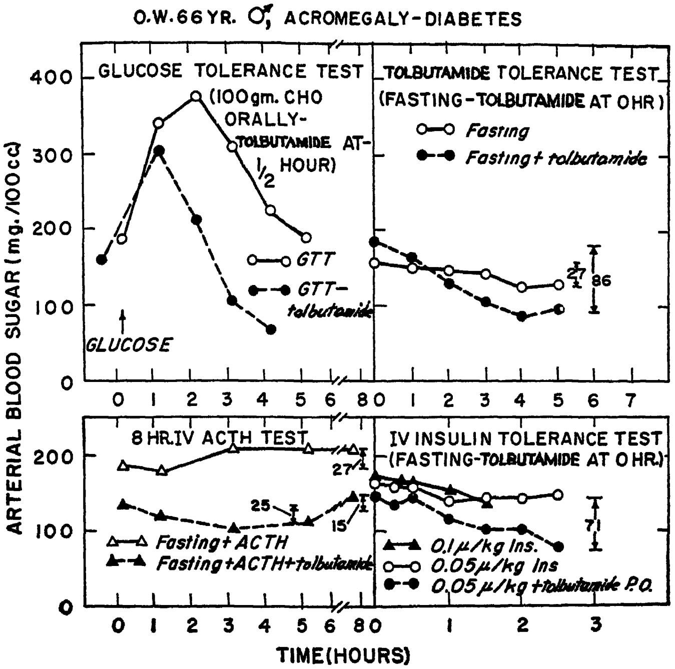

Figure 11

decrease in blood sugar was 86 mg. per cent at the end of 5 hr. (FIGURE 11). The insulin tolerance test showed an insulin-resistant type of response and, when 2 gm. of tolbutamide was given orally at the same time as insulin, no greater decrease in blood sugar than that observed during the fasting tolbutamide test was noted.

During the intravenous administration of 20 units of ACTH over an 8-hr. fasting period, the blood sugar rose 27 mg. per cent. However, when 2.5 gm. of tolbutamide was given orally at the beginning of the ACTH test, the blood sugar by the fifth hour had decreased 25 mg. per cent, but by the eighth hour was 15 mg. per cent above the initial blood sugar. Thus, the decrease of 80 mg. per cent observed during the fasting tolbutamide test was inhibited by the simultaneous infusion of ACTH; that is, the increase in the adrenocortical hormone production was capable of inhibiting the tolbutamide response. One possible explanation of this would be an increase in insulin resistance (FIGURE 11).

The oral glucose tolerance curve was quite different following the administration of 2.5 gm. of tolbutamide orally. The patient had what appeared to be a mild hypoglycemic reaction at the end of 4 hours, at a time when his blood sugar was 65 mg. per cent (FIGURE 11). In all the cases studied with oral glucose tolerance tests, this was the only one in which there was a significant difference between the control glucose tolerance curve and the glucose tolerance curve obtained after the administration of tolbutamide.

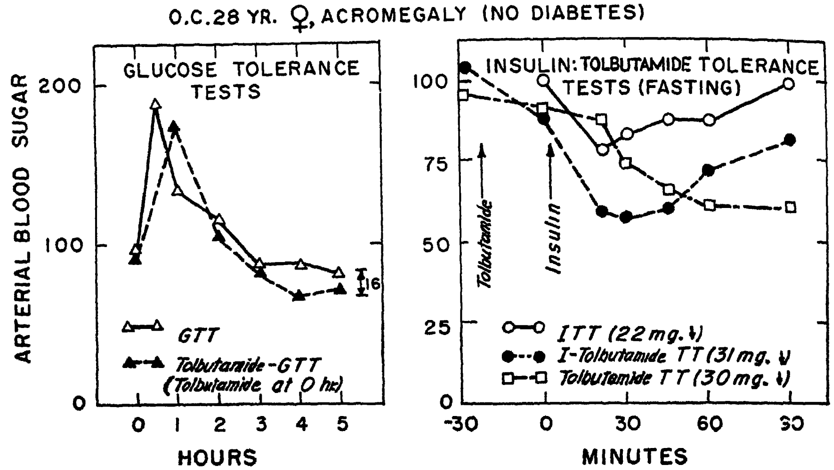

FIGURE 12

Patients with Acromegaly without Diabetes Mellitus

The patient, O. C., is a 28-yr.-old female with active acromegaly. There was no evidence of diabetes mellitus as shown by the oral glucose tolerance curve (FIGURE 12). No significant change in the oral glucose tolerance curve was noted when oral tolbutamide was given. The insulin tolerance test revealed a relative insulin resistance, and the fasting tolbutamide test showed a progressive decrease in blood sugar, with a maximum drop of 30 mg. per cent at the end of 2 hours. When insulin and tolbutamide were given together, as shown in FIGURE 12, the response was approximately the same as with insulin alone. Thus, the continuing progressive hypoglycemia seen during the fasting tolbutamide test was antagonized when insulin and tolbutamide were given together. This is similar to observations made in the rat by Lang and Sherry.[12]

Patients with Altered Endocrine States

Hypophysectomy was performed on 2 patients in an attempt to control metastatic carcinoma of the breast. These patients were maintained on 50 mg. of cortisone acetate, including the day of the tests, and at no time did they show evidence of hypoglycemia. Oral glucose tolerance tests were performed with or without the addition of 2 gm. of tolbutamide. One patient, H. M., a 73-yr.-old female who had had a hypophysectomy 2½ years before, had a mild diabetic glucose tolerance curve that was not altered by tolbutamide; the other, V. R., a 49-yr.-old female who had had a hypophysectomy 6 months before, had a relatively flat oral glucose tolerance curve that was not altered by administration of tolbutamide (FIGURE 13).

Two patients had had bilateral adrenalectomy, one for control of metastatic carcinoma of the breast and the other for metastatic carcinoma of the prostate. The female patient, M. W., who was maintained on 50 mg. of cortisone daily,

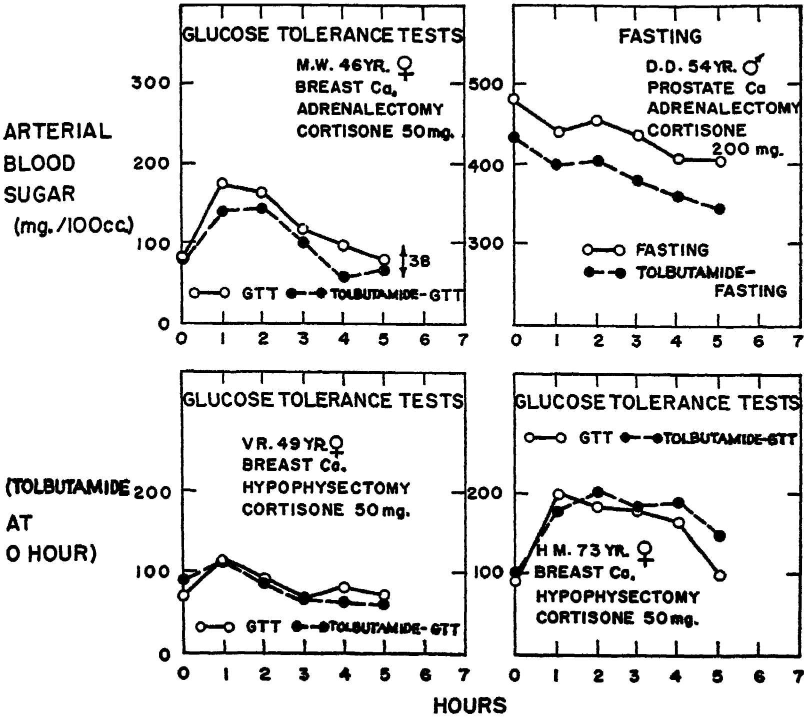

FIGURE 13. Glucose tolerance tests in adrenalectomized and in hypophysectomized patients.

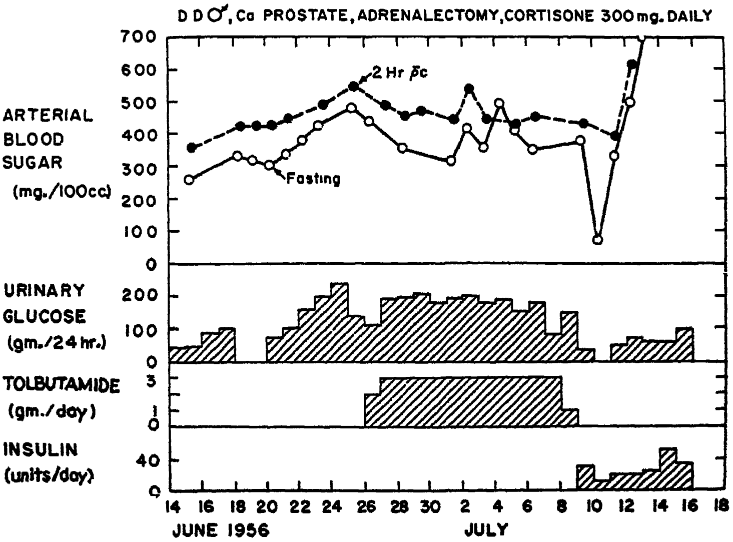

FIGURE 14

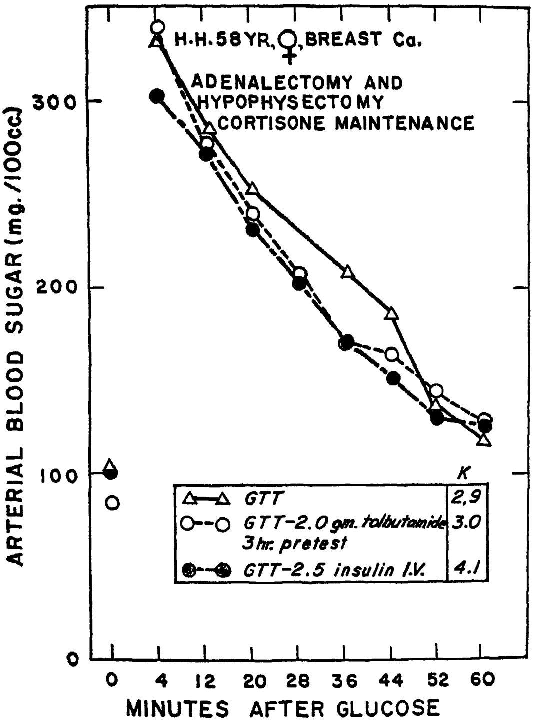

FIGURE 15. Glucose disappearance rates.

showed a slightly lower glucose tolerance curve following the oral administration of 2 gm. of tolbutamide (FIGURE 13). The essentially normal glucose tolerance curve, with failure to show increased sensitivity to tolbutamide that is shown by the adrenalectomized rat[22] is probably due to the cortisone replacement therapy. Similarly, a series of 50 cases in which oral glucose tolerance tests were performed before and after bilateral adrenalectomy and while the patient was on cortisone maintenance showed no difference in response to a glucose load.[23]

The patient who had adrenalectomy for cancer of the prostate had such severe bone pain that relief could be obtained only by increasing the cortisone to levels of 200 to 300 mg. a day. On these high doses of cortisone the patient developed a severe steroid diabetes. Despite the very high blood sugar, the fasting tolbutamide test produced a decrease in blood sugar of 85 mg. per cent, as compared to a decrease of 60 mg. per cent during the fasting test. This represents a minimal effect of the tolbutamide (FIGURE 13). When tolbutamide was given at a dose of 3 gm. a day, the blood sugars appeared to level off, and when the tolbutamide was stopped, despite the use of insulin, the blood sugars rose to very high levels. At this time, however, a septicemia had also developed. It is obvious that, at this dose level, tolbutamide was unable adequately to control the steroid diabetes of this patient (FIGURE 14).

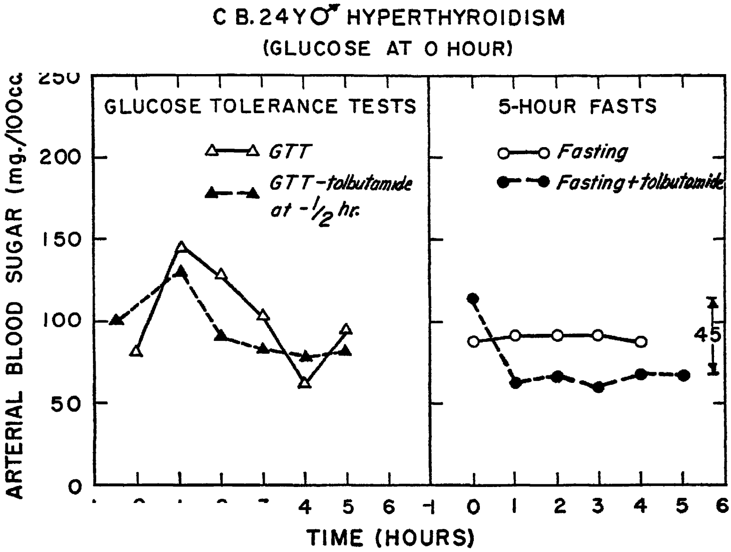

FIGURE 16

Patient H. H., a 58-yr.-old female with metastatic carcinoma of the breast, had had bilateral adrenalectomy and hypophysectomy and was maintained on 75 mg. of cortisone acetate daily. Three intravenous glucose tolerance tests were performed: one with glucose alone, one with 2 gm. of oral tolbutamide given 2 hours before the glucose, and another in which 2.5 units of insulin were given with the glucose. The glucose disappearance rates were determined; no significant difference between the control and the tolbutamide test was observed, while the insulin test showed an increase in glucose disappearance (FIGURE 15). This is further evidence that in this situation tolbutamide does not act as does exogenous insulin given intravenously.

C. B., a 24-yr.-old male with hyperthyroidism, was studied in an attempt to determine whether tolbutamide would block the uptake of radioactive iodine by the thyroid. Prior to tolbutamide, the 24-hr. uptake of radioactive iodine was 92 per cent and the B.M.R. was +27. The patient was placed on phenobarbital sedation for a week before tolbutamide administration was begun. Tolbutamide, 2.5 gm., was given orally daily for 6 days and, on the last day, the radioactive iodine uptake was 94 per cent and the B.M.R +18. There was no change in the clinical symptoms of his hyperthyroidism. The oral glucose tolerance and fasting tests with and without tolbutamide are shown in FIGURE 16. A point of interest is that the fasting curve was flat while, during the fasting tolbutamide test, there was a rapid drop in blood sugar in 1 hr. without subsequent decrease in blood sugar over the next 4 hr.

DISCUSSION

The data presented here do not reveal the mechanism or the site of action of tolbutamide; however, they do bring into focus the relationship of certain altered endocrine states to the hypoglycemic effect of tolbutamide.

The diabetes associated with acromegaly, as presented in two cases, was adequately controlled with oral tolbutamide. There did not appear to be any evidence that overfunctioning of the adrenal cortex was responsible for the diabetes or that tolbutamide produced any alteration in adrenocortical function that would account for its hypoglycemic action. Acromegaly is thought to be related to increased secretion of growth hormone by the pituitary, but one can only speculate as to whether tolbutamide inhibits the action of growth hormone or activates bound insulin. Until growth hormone can be measured in the blood or until an adequate supply of primate growth hormone is available to test its hyperglycemic action and whether this hyperglycemic action may be modified by tolbutamide, the problem will remain unanswered. All three of the acromegalic patients showed mild insulin resistance. However, the tolbutamide did not appear to increase the rate of disappearance of excess glucose following an intravenous glucose load. The blood phosphorus was not lowered during the administration of tolbutamide in these patients.

These data, together with reports by others,[24, 25, 26] would indicate that the intact pituitary-adrenal system is not necessary for the hypoglycemic effect of tolbutamide. Nevertheless, the level of circulating adrenal steroids may modify the degree of response to tolbutamide, as was revealed during the intravenous ACTH test in patient O. W.

There is no evidence that anti-insulinase activity of tolbutamide plays a significant role in the response observed in these patients, for there was no convincing evidence of potentiation of exogenous insulin.

The per cent rate of disappearance of excess glucose following tolbutamide administration did not approach the rate observed following intravenous administration of exogenous insulin. Our data do not indicate that tolbutamide, when given orally, is capable of stimulating to any considerable degree the secretion of endogenous insulin by the pancreas. However, the possibility of a prolonged secretion of a low level of endogenous insulin would be compatible with the observed results. Patient O. W. was the only subject in whom the oral glucose tolerance test seemed to be significantly altered by tolbutamide. These studies do not permit a statement as to the possible role of tolbutamide in decreasing hepatic glycogenolysis.

Summary

Nine patients with evidence of altered endocrine states have been studied in regard to the hypoglycemic effect of orally administered tolbutamide. Two patients with acromegaly associated with diabetes mellitus were studied while on a metabolic balance regimen, and the diabetes was controlled with the oral administration of 2 gm. of tolbutamide daily. The hypoglycemic response occurred without significant alteration in excretion of urinary 17-hydroxycorticoids and 17-ketosteroids. These findings would be compatible with an increase in insulin secretion, with activation of bound circulating insulin, or with decreased hepatic glycolysis.

The rate of disappearance of excess glucose with an intravenous glucose tolerance test following tolbutamide administration did not approach the

rate following intravenous insulin administration. This may be related to the route and rate of tolbutamide administration. Moreover, the effect on the release of endogenous insulin may be much less abrupt than a single intravenous dose of insulin and may produce a more prolonged release of insulin into the portal system.

Steroid-induced diabetes was not adequately controlled in one patient by oral tolbutamide.

The response to tolbutamide of 2 patients with adrenalectomy, 2 patients with hypophysectomy, and 1 patient with hypophysectomy and adrenalectomy maintained on cortisone, would indicate that the adrenal-pituitary system does not seem to be necessary for the action of tolbutamide.

Tolbutamide did not alter the elevated thyroidal uptake of I^{131} in a patient with hyperthyroidism.

Although the results of these studies do not permit a definition of the mode of action of tolbutamide, they provide observations that clearly point out the fact that tolbutamide may exert its hypoglycemic effect in the absence of, or without change in the function of, certain endocrine glands; however, the degree of the hypoglycemic response following tolbutamide may be altered with changes in the levels of certain hormonal substances in the body.

ACKNOWLEDGMENT

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CLINICAL EXPERIENCES WITH THE SULFONYLUREA COMPOUNDS

By Garfield G. Duncan, Charles T. Lee, and J. Kent Young
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In our work with sulfonylurea compounds our objectives were: a clinical study of their capacity for correcting glycosuria and hyperglycemia; the establishment of a simple means of detecting those patients who were likely to be and those not likely to be benefited by them; and the establishment of an optimum regimen of timing and dosage of the drug, with observation of effects, favorable or otherwise, after its prolonged use.

Determination of the exact mechanism, or mechanisms, by which sulfonylureas reduce glycosuria and hyperglycemia is the task of those equipped to do basic research on the problem. The critical clinician will not feel fully at ease in using these products and will continue to look askance upon them until these research problems are solved; until then he will view the compounds as necessary objects of research and not as products to be recommended for widespread clinical use.

Several methods have been used in the selection of patients favorably sensitive to carbutamide or tolbutamide. The first method proved satisfactory, but its completion required a minimum of 15 days. The study was divided into 3 consecutive 5-day periods, prior to which a reduction in the insulin was made to ensure that glycosuria and hyperglycemia would prevail during the control periods. Diet and exercise were constant. Quantitative determinations of the glycosuria for each 24 hr., and daily fasting and 2-hr. postprandial blood sugars were done. Following the initial 5-day control period, 1.0 gm. of either carbutamide or tolbutamide was given every 6 hr. throughout the second 5-day period. The drug was withdrawn during the final 5-day period. The results shown in FIGURE 1 reveal a daily average of 28.9 gm. of sugar in the urine in the first 5 days, 1.3 gm. daily while receiving the drug, and 9.8 gm. daily during the 5 days following its withdrawal. A favorable influence is also evident in the fasting and postprandial blood sugar values. Indeed, these effects carried over somewhat into the final 5-day control period.

In contrast to these results, FIGURE 2 illustrates the failure of a sulfonylurea compound to exert a favorable effect on the degree of glycosuria in a young patient with unstable diabetes. Indeed, as has been seen frequently in such a patient, the glycosuria during the final 5-day control period was increased appreciably over that of the first 5-day control period.

A second method of study afforded an opportunity for observing whether or not tolbutamide was effective, how quickly it was effective, and whether its effectiveness in patients favorably sensitive to it persisted for at least 8 hr.

In this study the diet was prepared in a single formula and was then divided into 12 equal amounts, 1 of which was consumed at 2-hr. intervals.

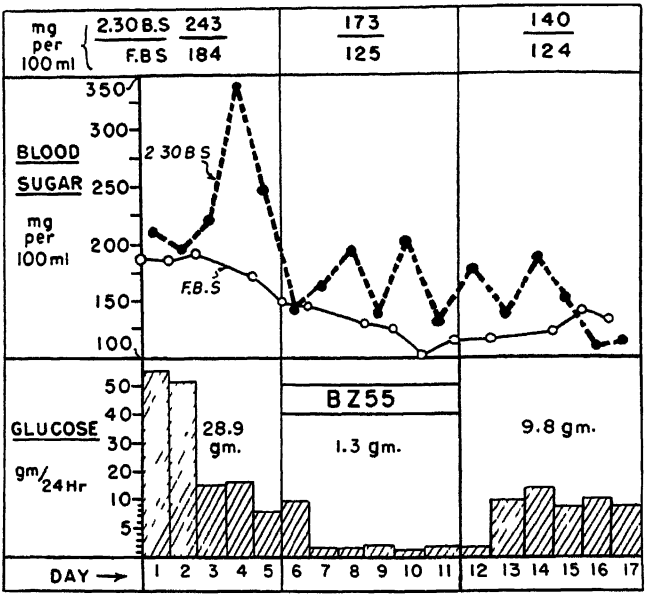

FIGURE 1. Favorable effect of carbutamide (BZ55) on the fasting and postprandial blood sugar values and glycosuria under controlled conditions. (After Duncan, Joiner, & Lee. 1956. Metabolism. 5: 964).

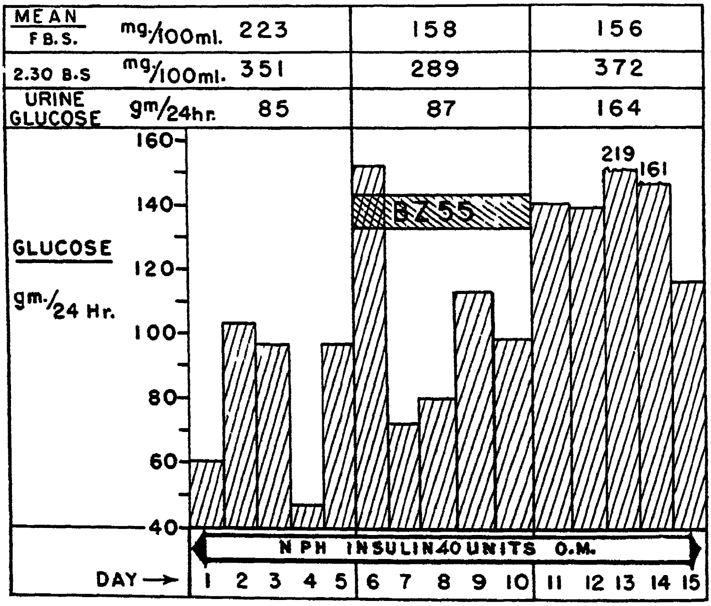

FIGURE 2. Failure of carbutamide (BZ55) to reduce the degree of glycosuria. In this case the glycosuria increased following a 5-day course of carbutamide.

The lowering of the blood sugar value in the face of these repeated feedings was more significant, I believe, than it would have been had the study been done with the patient in the fasting state. Also, the failure of the blood sugar to increase under this demand within 8 hr., gave some promise that the drug would be a practicable therapeutic agent.

In 2 cases, as indicated by the solid lines in FIGURE 3, 2 gm. of tolbutamide was without effect. Further observations with prolonged administration of the drug also failed to indicate any apparent effect in these 2 cases. This is not always so, however. The occasional patient who fails to respond favorably to an acute testing may, after some days or weeks of therapy, have a favorable response.

Prompt effectiveness of tolbutamide is indicated in the 2 cases represented by the broken lines. Prolonged therapy was equally effective, even during the period when the drug was reduced to 0.5 gm. twice daily.

A third method has proved least cumbersome and yet satisfactory in detecting the sensitivity of the patient to the therapeutic effectiveness of these drugs. The patient is admitted to the hospital the evening before the test and, on the following morning, the insulin is omitted, but the usual diet is given. After 7 A.M. each specimen of urine is examined for acetone. If, within 8 hr., appreciable amounts of ketonuria are found, as illustrated in FIGURE 4, no favorable effects may be anticipated from the sulfonylurea compounds. If no ketonuria occurs within 24 hr., however, a favorable response is probably certain, although the number of patients studied in this manner is still too small to permit a precise conclusion.

At least 1 type of case, 3 instances of which we have studied, is an exception to this response. In these 3 cases, each involving a patient over 40 years of age, the insulin requirement exceeded 100 units, and ketonuria developed

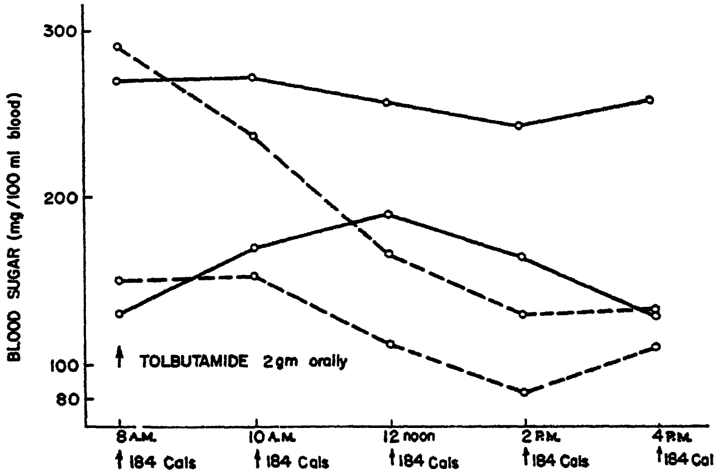

FIGURE 3. Effectiveness of tolbutamide in 2 cases (broken lines) and ineffectiveness in 2 other cases (solid lines). One twelfth of the day's total diet was given at 2-hr. intervals during the tests (after Duncan, Joiner & Lee. 1956. Metabolism. 5: 964).

MRS. M. W. AGE 53 HT. 68" WT. 163 LB.

DIABETES - 14 YEARS

DATE:	INSULIN (UNITS)	URINE			
3/14	18 NPH 14 REG.	SUGAR 2 ACETONE 0			
3/15	NONE IN A.M. 50 - REG. 10 P.M.	HOURS: SUGAR: ACETONE:	0 2 0	4 2 4	8 4 4

CARBUTAMIDE
0.5 GM. Q.I.D. - 3/20 - 4/24
RESULT: NO APPARENT EFFECT

FIGURE 4. The prompt appearance of a grade-4 ketonuria upon withdrawal of insulin, as illustrated, is an indication that the sulfonylurea compounds are not likely to be effective

promptly upon reduction of the dosage, although a great reduction of insulin was possible (FIGURE 5). Prior to the study, this patient required 140 units of insulin daily. When the insulin was reduced, first to 100 and then to 60 units, a large glycosuria ensued. The administration of carbutamide was followed by a reduction in the glycosuria, and a hypoglycemic reaction was encountered. After more than a year of sulfonylurea therapy (1 gm. daily) the need for insulin has increased to 84 units daily, but the diabetes appears to be more stable than it was prior to this treatment. This is true also in the other two such cases observed.

Observations have been made on 105 patients, a considerable number of whom were young, and in these cases the therapy was abandoned when a trial of a week or two revealed no favorable response.

Of 49 public-clinic patients given a therapeutic trial on an outpatient basis, 24 (49 per cent) experienced improved control of the diabetes while receiving carbutamide or tolbutamide. The elimination of insulin was tolerated without interfering with the control of the diabetes. There was some, but incomplete, response in 16 cases (33 per cent), and no response in 9 (18 per cent). Being regular attendants at a diabetic clinic, these 49 patients include a higher percentage with more severe diabetes than would be encountered in a cross section of the diabetic population.

Prompt control of the diabetes with one of the sulfonylureas was no guarantee that this state would continue indefinitely. Indeed, in at least one third of our patients glycosuria and hyperglycemia have returned after some months of uninterrupted therapy. It is not clear whether this has been due to the development of drug fastness, to the reduction of the dosage given, to the exhaustion of responsiveness to islet stimulation, or to decreased attention to the diet.

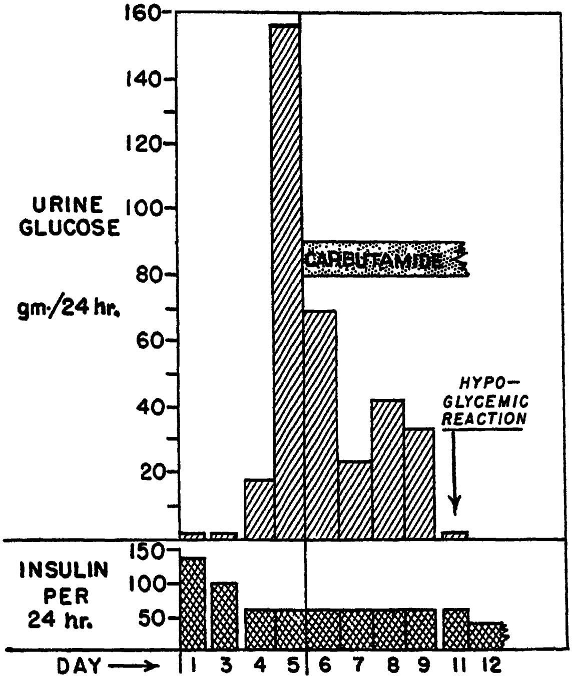

FIGURE 5. Prompt loss of large amounts of sugar in the urine occurred when the dosage of insulin was reduced from 140 units daily to 60 units. Without altering the dose of insulin, the addition of carbutamide was accompanied by an appreciable reduction of the degree of glycosuria. On the sixth day of this regimen, this patient had a hypoglycemic reaction.

Side effects were infrequent. One mild case of exfoliative dermatitis was observed following carbutamide therapy. After recovery, this patient had a recurrence of this disorder when tolbutamide was given. Urticaria occurred in one instance. In another case the drug was stopped because of abdominal pain; this neurotic patient had the same ill effect from lactose given in capsules. A seventh-nerve palsy was observed during tolbutamide therapy. While recovery was prompt following withdrawal of the drug, the cause and effect have not been established. No hematological abnormalities have been noted. This may be because we do not give more than 1 gm. of tolbutamide daily.

Changes in weight have not been great. Of 24 patients receiving tolbutamide, 8 have gained, and 9 have lost weight; in 7 there has been no change.

In conclusion, short-term tests of value in predicting responsiveness of patients to the sulfonylurea compounds have been presented. We realize that on the basis of these we may have eliminated from our studies some patients who might have derived benefit from prolonged therapy.

Of 105 patients from a clinic and private patient series, a favorable effect on the control of diabetes was observed in approximately two thirds. The side effects so far observed offer no real contraindication to continued long-term study of these compounds on a broad basis. They are not substitutes for insulin, nor are they effective in the presence of ketosis or acute infections. This does not deny them, however, if they prove harmless, a place in the treatment for uncomplicated diabetes.

At present, the only known advantage of these compounds over insulin is the convenience of oral administration. If we could assume that they are harmless, their ideal application would be in treating the obese diabetic who will not adhere to a reducing diet. In such cases, almost without exception, the treatment will control the diabetes but, in effect, may add to the degree of obesity, due to the cessation of glycosuria, unless some restrictions in diet are observed. The ideal treatment for the overweight diabetic—and this means the majority of diabetics—may prove to be a combination of undernutrition and sulfonylurea therapy. In embracing a new and convenient therapy, the advantages of reducing the excess weight should not be overlooked; if the new drug encourages a continuance of the obese state, its advantages will be questionable.

Tolbutamide has proved effective in controlling hyperglycemia and glycosuria. Its field of application may involve two thirds of those patients with "adult developed" diabetes. Its side effects appear minimal, but full evaluation of this aspect must await further experience. Favorable effects are minimal, or absent, in those patients who have a severe form of diabetes, and are greatest in those patients who do not need insulin and for whom we have, in the undernutrition treatment, a more logical therapy. An appropriate combination of these measures—restricted caloric intake supplemented by a sulfonylurea compound—may provide a more favorable outlook for patients with mild diabetes. Much will depend, however, on whether or not apparent benefit is maintained indefinitely.

CLINICAL EXPERIENCE WITH SULFONYLUREA COMPOUNDS IN DIABETES*

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Our experience with carbutamide and tolbutamide will be summarized from the standpoint of degree of control of hyperglycemia and glycosuria, untoward effects and, as regards carbutamide, the effect of long-term administration on liver-function tests. In addition, our findings in children given a single dose response test will be presented and the results correlated with duration of diabetes.

Clinical Material

Carbutamide. Our experience with carbutamide (BZ-55) began in December 1955; from that time until the present we have used this preparation with 450 selected patients with diabetes. Of this total number, maintenance studies have been carried out in 209 persons who have received the drug for 2 weeks or longer (TABLE 1). Since its withdrawal from clinical investigation in October 1956, use of the preparation has been discontinued in almost all patients. Whenever possible, certain liver-function tests were carried out before finally stopping the drug. At present only 31 patients are receiving carbutamide, and in no case is the dose larger than 1 gm. daily.

TABLE 1
EXPERIENCE WITH SULFONYLUREA COMPOUNDS

	No. of patients	
	Carbutamide	Tolbutamide
Maintenance studies	209	195
Response test only	195	195
Insufficient data	16	27
Discontinued because of untoward effects	27	3
Discontinued for other reasons	3	—
	450	420

In 195 persons, carbutamide was given on a single occasion at the time of a response test designed to ascertain the degree of fall of blood sugar in 4 hr. (TABLE 1). Details concerning this response test have been published pre-

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* The study reported in this paper was aided by grants from The Upjohn Company, Kalamazoo, Mich., Eli Lilly & Company, Indianapolis, Ind. and the Warner-Chilcott Laboratories, Morris Plains, N. J.

† Holder of a foreign fellowship, Eli Lilly & Company, Indianapolis, Ind.

TABLE 2

LENGTH OF TREATMENT WITH SULFONYLUREA COMPOUNDS

Months	No of P	
	Carbutamide	Tolbutamide
0–3.9	27	44
4–7.9	51	93
8–11.9	107	58
12–15.9	24	
	209	195

viously.[1, 2] Findings in 16 patients maintained on carbutamide for less than 2 weeks have been excluded. In 30 patients the compound was discontinued, chiefly because of untoward effects. These data are shown in TABLE 1. The length of time patients were maintained on the drugs is shown in TABLE 2. It will be noted that 107 patients took carbutamide for 8 to 12 months, and 24 for 12 to 16 months.

Tolbutamide. Our experience with tolbutamide (Orinase) extends from the last days of February 1956 until the present. During that time 420 selected patients have received this compound. As indicated in TABLE 1, 195 patients have been maintained on the preparation for periods ranging from 2 weeks to a year. A response test to a single dose was carried out in an additional 195 patients who were not maintained on the drug. Twenty-seven patients were excluded from the studies because of insufficient data and, in 3, the preparation was discontinued because of untoward effects (TABLES 1 and 2).

Results of Maintenance Studies

Criteria of control. In order to evaluate the degree of control obtained with the sulfonylurea compounds, certain standards were chosen arbitrarily. These have been published before,[1, 2] but are repeated here in detail for the sake of clarity (TABLE 3). Briefly, however, they provide that control will be considered "good" if 70 per cent or more of "true" blood sugar values at 3 or more hours after food are 110 mg. per 100 cc. or below, and if the urine contains 2 gm. or less of sugar in 24 hours. For control to be judged as "fair," 70 per cent or more of "true" blood sugar values at 3 or more hours after meals must be 130 mg. per 100 cc. or below, and the amount of sugar in the urine must be 5 gm. or less in 24 hours. Failure to meet these standards constitutes "poor control." As indicated in TABLE 3, standards for blood sugar levels at 1 and 2 hours after food have also been set up.

Degree of Control Secured

Carbutamide. Using the standards just described, 61 per cent of patients maintained on carbutamide were found to have good control of hyperglycemia

TABLE 3
STANDARDS OF CONTROL*

Relation to food	Degree of control† Good		Fair		Poor
	Blood sugar‡ (mg./100 cc.)	Urine sugar (%)	Blood sugar‡ (mg./100 cc.)	Urine sugar (%)	
Fasting	110	Trace	130	0.1	All other cases
1 hr. p.c.	150	0.3	180	0.5	
2 hr. p.c.	130	0.1	150	0.3	
3 hr. p.c.	110	Trace	130	0.1	
Urine sugar in 24 hr.	2 gm. or less		5 gm. or less		

* For the purpose of classification as to degree of control 70 per cent or more of the values must conform with the standards listed in the table.

† These standard values are the highest acceptable.

‡ Glucose as determined by the Somogyi-Nelson procedure.

and glycosuria. Nine per cent had fair and 30 per cent poor control (TABLE 4).

Tolbutamide. Of 195 patients maintained on tolbutamide for 2 weeks or more 52 per cent achieved good, 14 per cent fair, and 34 per cent poor control.

It is evident from a combination of the figures for good and fair control that 70 per cent of the patients on carbutamide and 66 per cent on tolbutamide could be considered to have achieved reasonably good success, particularly since the criteria as outlined in TABLE 3 are strict in comparison with the usual clinical standards employed with patients receiving insulin. How-

TABLE 4
RESULTS OF MAINTENANCE STUDIES

Results	Carbutamide Patients No.	%	Tolbutamide Patients No.	%
Good	128	61 } 70%	102	52 } 66%
Fair	19	9	28	14
Poor	62	30	65	34
	209	100	195	100

ever, it is probably significant that the percentage of patients achieving good control was greater in the case of those on carbutamide. It is our impression that this compound is somewhat more hypoglycemic than tolbutamide.

In 68 patients who had achieved good control of hyperglycemia while on carbutamide, the drug was discontinued. In 21 (or 31 per cent) of these the blood sugar remained at a normal level. A similar experience was reported following the discontinuance of tolbutamide in 31 patients who had achieved good control of hyperglycemia. In 12 (39 per cent) of these the blood sugar remained at or near the normal level. This experience emphasizes the fact that, in evaluating results of treatment with the sulfonylurea compounds in middle-aged or elderly patients, due regard must be given to the effect of dietary restriction and long-continued control. However, the fact that in this small series 69 per cent of the patients on carbutamide and 61 per cent of those on tolbutamide experienced a rise in the blood sugar level following discontinuance of the compounds emphasizes the active hypoglycemic character of these drugs.

Untoward Effects

Listed in TABLE 5 are the untoward effects seen in patients receiving carbutamide and tolbutamide. Among 328 patients on carbutamide, there were 37 (9 per cent) in whom untoward sequelae were seen. It should be mentioned that among the 5 patients listed with "other" complications there were 2 in whom cerebral vascular accidents occurred and 1 who suffered an attack of paroxysmal auricular tachycardia. It is extremely doubtful that these occurrences can be attributed to the sulfonylurea compound. The frequency of untoward effects was definitely high, however, particularly since they included 4 instances of jaundice. One of these patients died, as has been previously reported.[1] This experience has re-emphasized to us the

TABLE 5
UNTOWARD EFFECTS*

	No. of patients	
	Carbutamide	Tolbutamide
Skin rash	19	2
Nausea and/or vomiting	5	
Diarrhea	2	1
Jaundice	4	
Prolonged hypoglycemia	2	
Other complications	5	
Number	37	3
Per cent of total	9	0 9

* These data concern 328 patients on carbutamide and 314 on tolbutamide. From these were excluded 122 children on carbutamide and 106 on tolbutamide.

necessity for making certain that the oral hypoglycemic agents are not hepatotoxic.

Although tolbutamide is less hypoglycemic than carbutamide, experience to date indicates that it is less toxic, as brought out in TABLE 5. Only 3 of 314 patients experienced untoward effects, an incidence of only 0.9 per cent. From a clinical standpoint, no serious complication has been encountered thus far in patients receiving tolbutamide.

No instance of leukopenia was encountered in patients taking either compound. In one patient on carbutamide who developed jaundice, moderate anemia occurred. Recovery took place on discontinuance of the drug.

Liver-Function Studies

Because of the instances of jaundice we have encountered and because of the possibility that the sulfonylurea compounds might exert their effect by action on the liver, late in the fall of 1956 we began to carry out liver-function tests with patients on carbutamide. Unfortunately, no control or base-line studies had been done on these patients. In order that our retrospective study might have significance, we have made use of data collected by Bradley, Sagild, and Schertenleib of our group,[3] and have carried out similar liver-function tests on 44 diabetic patients of comparable age, sex, and duration of diabetes. Both in the series of Bradley *et al.* and in our recently studied group, no patient with frank liver disease has been used as a control, although subjects have not been excluded simply on the basis of a palpable liver.

The liver-function tests included in the study were the following: van den Bergh, 1-min. direct, indirect, and total; thymol turbidity and flocculation

TABLE 6
RESULTS OF LIVER-FUNCTION TESTS

Type of subject	No. of subjects	van den Bergh			Thymol		Cephalin flocculation		Bromsulfalein	Alkaline phosphatase
		1 min direct	Indirect	Total	Turb	Floc.	24-hr	48-hr.		
Normal limits.		0 4	0 6	1 0	4	2+	2+	2+	5	4 8
Percentage of subjects with abnormal results										
Bradley control series	118	0 8	—	6 8	0 8	0 8	2 5		10 8	Not done
Present control series...	44	0	0	2	7	0	0	0	12	15
Carbutamide series....	81	0	0	1	9.5	1	1	6	26	30

tests; cephalin flocculation test, with reading at 24 and 48 hr.; bromsulfalein (BSP) test, using 5 mg. of dye per kg. of body weight and taking the final blood sample at 45 min.; and alkaline phosphatase (Bodansky).

In TABLE 6 are shown the values chosen as the upper limit of normal with each test, and the percentage of abnormal values found (1) in the Bradley control series, (2) in the present control series, and (3) in the carbutamide series of 81 patients. It is evident that the findings in the van den Bergh test showed less deviation from the normal in the carbutamide series than in the two control series. In the thymol tests there was no significant difference between the incidence of abnormal values in the carbutamide series as compared with the present control series. There was a slightly higher incidence of positive cephalin flocculation tests at 48 hr. in the carbutamide series as compared with the control series, but this was not striking. The chief interest centered, therefore, about the results of the bromsulfalein and the alkaline phosphatase tests. Regarding the former, 26 per cent of the patients in the carbutamide series showed abnormal results, as compared with 10.8 per cent in the Bradley control series and 12 per cent in the present control series. In the alkaline phosphatase test, 30 per cent of the carbutamide patients showed abnormal results, as compared with 15 per cent in the present control series. Alkaline phosphatase tests were not included in the studies of Bradley *et al.*

The data were analyzed further as to the percentage of patients with abnormal liver function tests after varying periods of time: up to 4 mo., 4 to 8 mo., 8 to 12 mo., and 12 to 16 mo. The results are shown graphically in FIGURES 1 and 2.

From FIGURE 1 it is evident that there was a tendency toward progressive impairment of function with the passage of time, as seen in the results of the bilirubin, thymol turbidity, and cephalin flocculation tests. However, the

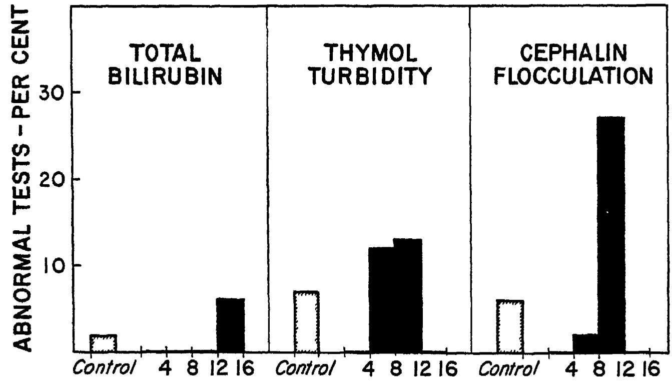

FIGURE 1. Percentage of patients with abnormal results in liver-function tests following the administration of carbutamide for periods up to 16 months. Note that, when compared with the results in control subjects, no important differences are apparent except in the cephalin flocculation tests carried out in patients who had taken carbutamide for 8 to 12 months.

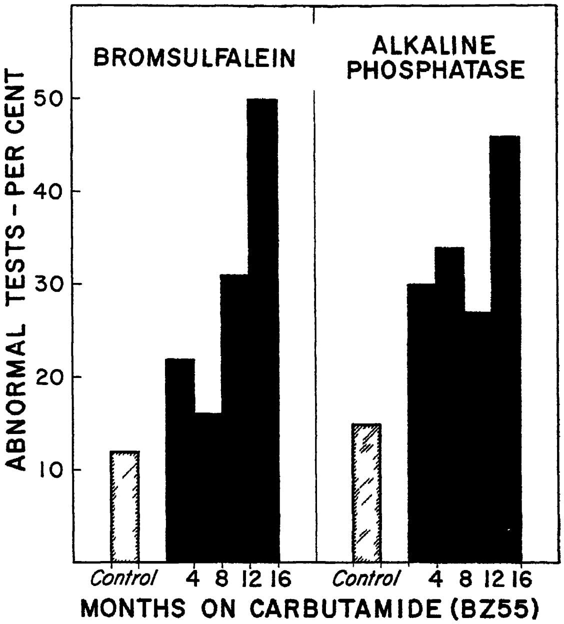

FIGURE 2. Results of bromsulfalein and alkaline phosphatase tests of liver function in patients receiving carbutamide up to 16 months Note that with long-continued administration there is a tendency toward an increasingly higher percentage of persons with abnormal findings.

results were somewhat erratic; for example, in the cephalin flocculation test, 27 per cent of 24 patients who had received carbutamide for 8 to 12 mo. had abnormal tests, whereas no abnormal findings were seen in 14 patients who had received the drug for 12 to 16 mo.

The analysis of data obtained in the bromsulfalein and alkaline phosphatase tests (FIGURE 2) yielded findings that appear to be of more significance. It is evident that there was a definite tendency toward a higher percentage of abnormal results in patients who had received carbutamide for longer periods of time. The patients tested included 22 who had received this drug for 8 to 12 mo. Among this group 7 (31 per cent) had abnormal bromsulfalein tests, and 6 (27 per cent) had abnormal alkaline phosphatase tests. Of the patients who had received the drug for 12 to 16 mo., 15 were studied with the alkaline phosphatase test and 14 with the bromsulfalein test (1 patient did not have the latter test). In each instance there were 7 patients with abnormal results, or 46 per cent in the alkaline phosphatase, and 50 per cent in the bromsulfalein test.

From these results it would appear reasonable to conclude that in the long-term administration of carbutamide, even in low dosage, a significant percentage of individuals experience impairment of liver function, at least insofar as such impairment is reflected in bromsulfalein retention and in elevation of alkaline phosphatase. On further study, however, one finds that the situation is less straightforward than would appear at first. Thus, when we examine in detail in individual patients the trend of results with continued administration of the drug, we find that in certain patients, with the passage of time, even though abnormal findings were obtained several months after the beginning of treatment, the degree of abnormality does not rise; in fact, it might fall or a normal condition might be attained. The effect is not unlike that reported recently by Dickes *et al.*[4] in studies of patients receiving chlorpromazine over long periods of time. One can only speculate as to whether this represents an adaptation on the part of the liver toward a foreign agent.

Similar studies of liver function are in progress in patients who have received tolbutamide. These data are as yet incomplete, particularly since there are available relatively few patients who have had the compound for more than 9 months. The results will be reported at a later date.

Mode of Action of Sulfonylurea Compounds

In an attempt to throw light on the question as to the site and mode of action of the sulfonylurea drugs, studies were carried out on 228 children with diabetes of varying duration. The children were given single doses of 1.5 to 3.0 gm. of carbutamide (122 cases) or tolbutamide (106 cases) in the fasting state, and the capillary blood sugar was determined before and at 2 and 4 hr. after administration of the sulfonylurea compound. On the basis of previous experience,[1, 2] a fall of blood sugar at 4 hr. of more than 20 per cent, when compared with the initial value, was considered a satisfactory result.

The data have been reported in detail elsewhere,[5] but the correlation between the percentage of patients with a satisfactory fall in blood sugar and the duration of diabetes is shown in FIGURE 3. It is striking that, of 10 patients with duration of diabetes of less than 6 months, 9 (90 per cent) showed a satisfactory fall. Of 11 patients with duration of diabetes of 6 to 12 months, 63 per cent showed a satisfactory fall. In contrast to this, of 90 patients with diabetes of 5 or more years' duration, only 6 per cent showed a satisfactory fall in blood sugar.

It is generally accepted that patients with juvenile diabetes of 5 or more years' duration possess little or no capacity for the production of insulin. On the other hand, in children with diabetes of recent onset, temporary remissions occur occasionally, thus indicating some capacity for insulin production at that stage of the disease. Therefore, it would appear reasonable to postulate that the sulfonylurea drugs in single doses are hypoglycemic in children with diabetes of recent onset, and are ineffective in children with diabetes of long duration because, in the former group, a pancreas capable of at least partial function is present.

Summary

(1) Experience is reported with 450 selected patients with diabetes who received carbutamide and 420 who received tolbutamide. Of these, 209 were maintained daily on the former and 195 on the latter for periods ranging from

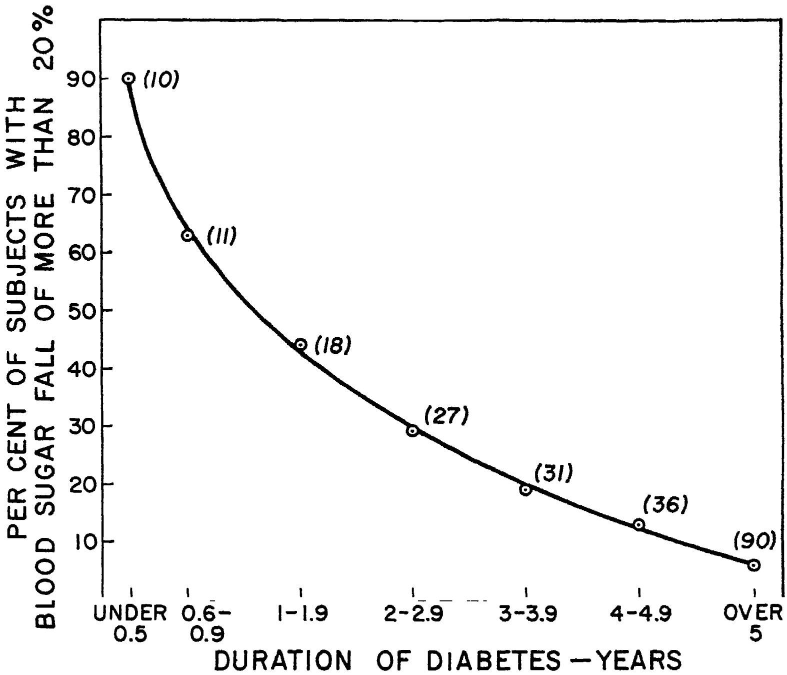

FIGURE 3. Relationship of fall in blood sugar to duration of diabetes, as determined in 223 children 4 hours after a single dose of carbutamide or tolbutamide. The figures in parentheses indicate the numbers of subjects at each interval of duration of diabetes. Thus, 90 per cent of 10 children with a duration of diabetes of less than six months showed a fall in blood sugar of more than 10 per cent, whereas only 6 per cent of 90 children with a duration over 5 years responded positively (from Marble[6]).

2 weeks to 16 months. Maintenance for 8 months or longer was carried out in 131 patients with carbutamide and in 58 patients with tolbutamide.

(2) Good control of hyperglycemia and of glycosuria was obtained in 61 per cent of selected patients on carbutamide. In an additional 9 per cent the degree of control was fair. The corresponding figures for those patients on tolbutamide were 52 and 14 per cent, respectively.

(3) The incidence of untoward effects was 9 per cent in 328 patients receiving carbutamide and 0.9 per cent in 314 patients receiving tolbutamide. Four patients on carbutamide developed jaundice, and one of these died. No instance of leukopenia or agranulocytosis was encountered.

(4) Certain liver-function tests were carried out in patients who had received carbutamide for long periods of time. The chief abnormalities were noted in the bromsulfalein and alkaline phosphatase tests. Twenty-six per cent showed abnormal bromsulfalein retention as compared with values of 10.8 and 12 per cent, respectively, in two control series. In the alkaline phosphatase test, 30 per cent of the carbutamide patients showed abnormal results, as compared with 15 per cent in the control series. There was a tendency to

an increasingly greater incidence of abnormal results with continued administration of the drug.

(5) In response tests to a single dose of both compounds carried out in 228 children with diabetes, it was found that, of 10 patients with duration of diabetes of less than 6 months, 90 per cent showed a satisfactory fall in blood sugar level. In contrast to this, only 6 per cent of those with duration of diabetes of 5 or more years showed a satisfactory response. These findings appear consistent with the thought that the presence of insulin, or of a pancreas capable of producing some insulin, is necessary for the hypoglycemic effect of the sulfonylurea compounds.

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THE USE OF TOLBUTAMIDE IN THE MANAGEMENT OF ADULT DIABETES*

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It is the purpose of this report to describe our clinical experience with tolbutamide (Orinase) in the management of the adult diabetic patient.

Methods and Results

Observations have been made on 75 adult diabetics, ranging in age from 23 to 74, who have been maintained on oral therapy for periods of 1 month to 1 year. Figure 1 describes the duration of tolbutamide therapy in the patients under study. It will be noted that therapy has been discontinued in 11 patients: 9 within the first month; 1 at the end of 3 months; and 1 at the end of 9 months. Three patients have been lost to follow-up: 1 during the first month, and 2 during the third month. There are 61 patients still on tolbutamide therapy. The average duration of observation in this group is

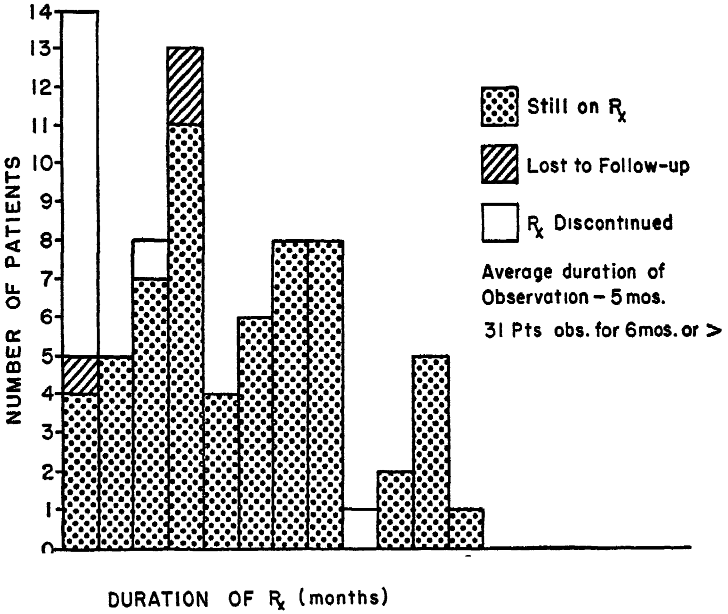

Figure 1. Duration of tolbutamide therapy in 75 patients with diabetes mellitus.

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* The work on which this paper is based was supported in part by a grant from The Upjohn Company, Kalamazoo, Mich. The tolbutamide used in this study was Orinase, from The Upjohn Company. Our preliminary observations with this agent were reported in *Metabolism*, vol. 5, p. 911, 1956.

TABLE 1
RESULTS OF TOLBUTAMIDE THERAPY IN 75 ADULT DIABETICS

Response	Initial		Long-term[*]	
	No	%	No	%
Good	51	68	40	56
Partial	13	17	18	25
None	11	15	14	19

[*] 3 patients lost to follow-up.

6 months, but 31 of the patients have been observed for 6 months or longer. Some of the patients were originally observed in the hospital, but subsequently have been followed as outpatients, while the rest have been observed as outpatients only. All of the patients have been maintained on constant dietary regimens.† At the time of referral, 41 of the patients had been previously treated with diet alone, 31 with diet plus insulin, and 3 had been newly discovered. In the patients receiving insulin, the daily dosage varied from 8 to 44 units, with an average daily dose of 17 units. The status of the control of diabetes in the entire group of patients has been closely followed by frequent measurements of glycosuria and of the fasting and 2-hr.-post-breakfast blood sugar level. Additional studies on each of the patients have included the response to a screening dose of 1 gm. of sodium tolbutamide intravenously, and periodic repetition of a variety of laboratory tests designed for toxicity screening purposes.

No fixed pattern of tolbutamide therapy was employed. In most instances the entire daily dose of tolbutamide was given as a single dose before breakfast. Most patients were treated and then maintained on 1 or 2 gm. of tolbutamide daily. In instances where little or no response was noted, larger doses, from 3 to 6 gm. daily, were employed.

Clinical observations. Our results are summarized briefly in TABLE 1. It will be noted that the effects of tolbutamide therapy have been evaluated in terms of the initial response, as well as the response to prolonged therapy. The benefit derived from tolbutamide therapy has been arbitrarily classified as good, partial, or none. The patient's response has been considered good when one of the following phenomena were observed: a disappearance of the insulin requirement in the insulin-treated patients; or a disappearance of glycosuria with an associated fall in blood sugar in the non-insulin-treated glycosuric patients; or a marked fall in the fasting blood sugar toward normal levels in the aglycosuric patients. Using these criteria, which relate entirely to the control of the hyperglycemic and glycosuric aspects of the diabetes, it will be noted that initially 68 per cent of this group had a good response, 17 per cent a partial or fair response, and 15 per cent a poor response.

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† Most of the patients were maintained on a 2000-calorie diet consisting of 250 gm. carbohydrate, 90 gm. protein, and 70 gm. of fat daily.

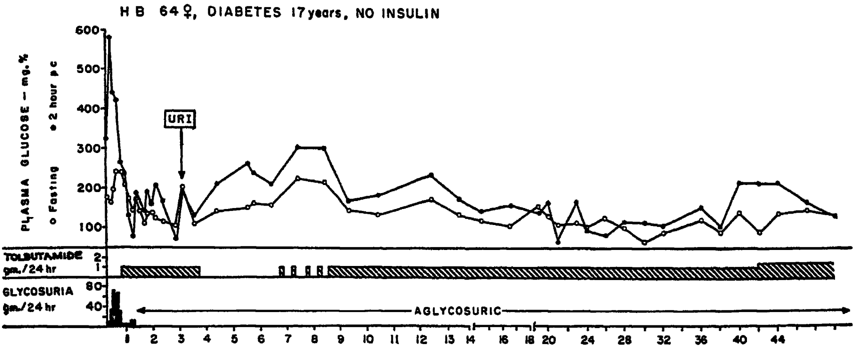

Figure 2

Also shown in table 1 is the evaluation of this group at the present time. Three patients have been excluded, since they have been lost to follow-up. Patients who were failures initially and had their therapy discontinued are included as long-term failures. It will be noted that 56 per cent still maintain a good response, 25 per cent now show a fair response, and 19 per cent are failures. Analysis of the data reveals that approximately 90 per cent of the patients with a good or fair initial response have maintained their response over the entire period of observation. In the remaining 10 per cent, the patients have not maintained as good a response or have become unresponsive to oral therapy.

An example of a good long-term response is illustrated in figure 2, in which are shown observations on patient H. B., a 64-year-old female with known diabetes of 17 years' duration, but without previous insulin therapy. It will be noted that initially, during a 1-wk. control period, the fasting plasma glucose level ranged between 175 and 250 mg. per cent, and the 2-hr. postprandial values ranged from 320 to 578 mg. per cent. Immediately upon institution of 1 gm. of tolbutamide daily, the postprandial values fell precipitously, followed shortly thereafter by a fall in the fasting plasma glucose and a diminution and then disappearance of the glycosuria. Three weeks following the institution of therapy, the fasting plasma glucose was maintained at approximately 110 mg. per cent, with a postprandial level at about 150 mg. per cent. Tolbutamide therapy was then discontinued. Over the next 3 weeks, the fasting glucose level rose significantly, as did the postprandial level. Intermittent tolbutamide therapy, 1 gm. twice weekly, was tried without effect. In the middle of the eighth week of observation, the patient was again placed on daily tolbutamide therapy. The fasting and postprandial blood sugar levels promptly fell, and then were maintained at lower levels. At about the thirty-ninth week of observation, it was noted that the postprandial level was beginning to rise, and the tolbutamide intake was increased to 1.5 gm. daily. On this regimen, the postprandial level fell. At present the patient has been observed for a full year and remains in good control.

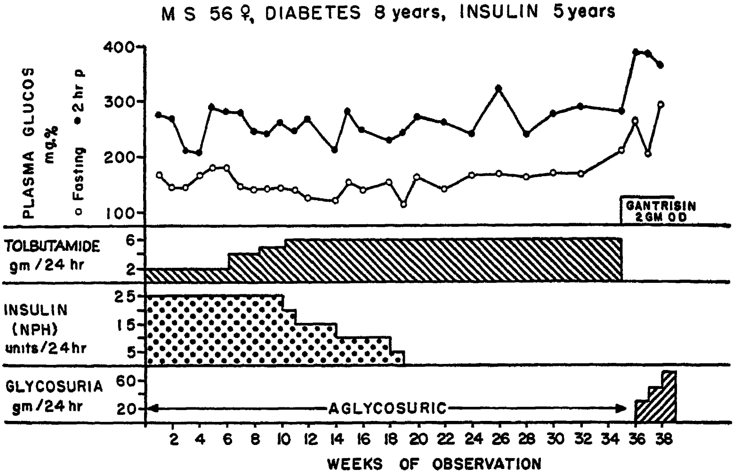

FIGURE 3

The beneficial effects ascribed to tolbutamide therapy in approximately 80 per cent of the patients under observation may be subjected to considerable criticism. It is appreciated that this is a select group, with 60 per cent of the patients over the age of 50; that the criteria employed are purely arbitrary and do not truly quantitate the effect of tolbutamide therapy; and that there is no way of estimating to what extent the renewed interest in the patients has led to better control other than through the specific agent under evaluation. For this reason, 7 patients who had been under observation on continuous tolbutamide therapy for 6 months or longer, and in whom good control was maintained, were selected for a trial on placebo therapy. Gantrisin (N^1-(3,4-dimethyl-5-isoxazolyl)-sulfanilamide) was used as the placebo. Of these 7 patients, 5 had an unequivocal deterioration in their control within 3 weeks of the placebo substitution, and tolbutamide therapy has been instituted again. The other 2 patients are still being observed on the placebo. FIGURE 3 illustrates the course in one of the patients who did poorly on the placebo.

Patient M. S. (FIGURE 3) is a 56-yr.-old female whose diabetes was diagnosed 8 years ago and who has received insulin therapy for 5 years. Prior to oral therapy, the patient was receiving 25 units of NPH insulin daily. On this regimen she was aglycosuric, with a fasting plasma glucose ranging from 150 to 175 mg. per cent, and a postprandial level of 225 to 275 mg. per cent. Tolbutamide was added to her previous regimen in increasing doses without significantly altering the blood sugar. After 10 weeks the insulin intake was successively reduced, and it was completely discontinued in the nineteenth week. For the next 16 weeks the patient was maintained on tolbutamide alone, at about the same level of control she had previously enjoyed with insulin. After a total of 35 weeks of therapy with tolbutamide, it was discontinued and Gantrisin was substituted. The fasting plasma glucose rose promptly, as did the postprandial levels. Significant glycosuria appeared for the first time. After 3 weeks on Gantrisin, the fasting plasma glucose had risen to 290 mg. per cent, her postprandial level was 365 mg. per cent, and she exhibited a 24-hr. glycosuria of 70 gm. Tolbutamide therapy has been reinstituted.

Intravenous sodium tolbutamide screening test. On the basis of preliminary observations we suggested that the response to a 1-gm. intravenous dose of sodium tolbutamide might prove to be a useful screening procedure in predicting the future response to oral therapy. In contrast to nondiabetics, who had a prompt fall in blood sugar in the first 30 minutes, followed by a return to normal by the end of the second hour, diabetics responded to the intravenous injection of tolbutamide with a slow and progressive fall in blood sugar of several hours' duration. Preliminary data suggested that diabetics who had a positive response to the sodium tolbutamide test—that is, a fall in blood sugar of 15 per cent or greater in 2 hours—responded to oral therapy, whereas diabetics who had less than a 15 per cent fall did not respond to subsequent oral therapy.

Further experience with this test has shown that the procedure has limited value. In general, patients with a significant fall in blood sugar following sodium tolbutamide intravenously were more likely to respond to oral therapy, but in a significant number of patients the prediction of the test was inaccurate. Furthermore, there was no correlation between the daily dose required to maintain a response in the patient and the sensitivity to the intravenously administered sulfonylurea. The factors responsible for the lack of correlation between the intravenous sensitivity and the clinical response are not apparent. That absorptive difficulties cannot be the sole explanation is illustrated by the data obtained in patient D. M.

Patient D. M. (FIGURE 4) is a 34-yr.-old female with known diabetes of 3 years' duration who refused to take insulin. During one week of control observations the fasting blood sugar ranged from 270 to 300 mg. per cent, and there were wide fluctuations in the postprandial levels. Glycosuria averaged over 100 gm. daily. On 3 gm. of tolbutamide daily she responded with a slight reduction in glycosuria, but the plasma glucose levels fluctuated widely. Because she proved to be sensitive to I.V. tolbutamide, her daily

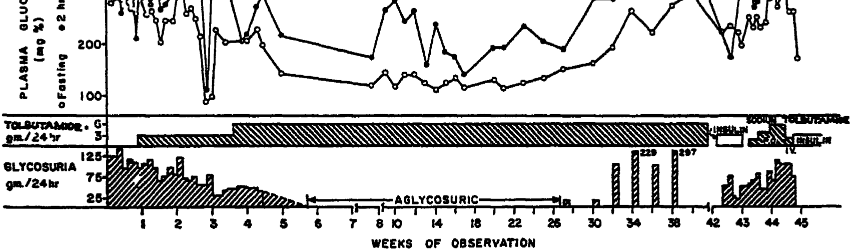

FIGURE 4

dose was raised to 6 gm. Shortly thereafter the fasting blood sugar fell to 140 mg. per cent, and her glycosuria disappeared. For the next 21 weeks the patient remained aglycosuric and with the fasting blood sugar well controlled. However, considerable fluctuations in the postprandial levels persisted. During the twenty-seventh week, despite continued tolbutamide therapy, the blood sugar began to rise, and glycosuria returned. By the fortieth week the patient was excreting over 200 gm. of glucose daily, and the fasting blood sugar was approximately 300 mg. per cent. During the forty-second week, tolbutamide was discontinued and insulin instituted for the first time. The fasting plasma glucose fell rapidly, and glycosuria rapidly diminished. Since the patient was still sensitive, by test, to intravenously administered sodium tolbutamide, she was taken off insulin and given large doses of sodium tolbutamide. The sodium salt was given, first orally and then intravenously, but it proved to be ineffective in controlling the hyperglycemia or glycosuria. With reinstitution of insulin therapy, there was a prompt fall in blood sugar and glycosuria.

Relation of response to previous diabetic state. Analysis of the results obtained in this series of patients reveals that, of all the variables studied (age, age at onset of diabetes, duration of diabetes, previous insulin therapy, duration of insulin therapy, and previous diabetic state), the previous diabetic state of the patient correlates most closely with the results of tolbutamide therapy. Unstable diabetics—that is, diabetics who had recurrent bouts of acidosis or coma, or who were difficult to control by the usual measures because of large spontaneous fluctuations in their fasting or postprandial blood sugar levels responded poorly to tolbutamide therapy, whereas stable diabetics did well. This correlation is illustrated in TABLE 2. Of 61 patients categorized as stable diabetics, 94 per cent had either a good or partial response on long-term tolbutamide therapy. In contrast, of 11 diabetics who had been difficult to control prior to tolbutamide therapy, only 2 responded initially, and only 1 maintained a prolonged response to the oral agent.

Toxicity of tolbutamide. The evaluation of toxicity is presented below under three categories: complications probably attributable to tolbutamide;

TABLE 2
RESULTS OF LONG-TERM TOLBUTAMIDE THERAPY AS RELATED TO PREVIOUS DIABETIC STATE

Response	Stable diabetics (Total 61)		Unstable diabetics* (Total 11)	
	No.	%	No.	%
Good	39	64	1	9
Partial	18	30	0	0
None	4	6	10	91

* Unstable = previous bouts of ketosis or difficult to control by usual measures.

complications not associated with tolbutamide; and the results of the screening tests for hepatic, hematological, and renal dysfunction.

A number of minor complications that have been observed are probably attributable to tolbutamide. One patient had an urticarial reaction that occurred on the fourth day of therapy and promptly subsided upon withdrawal of tolbutamide. Three patients developed epigastric fullness. In only 1 of the 3 did the symptom promptly subside with the discontinuation of the medication. Factors other than tolbutamide may have been present in the other 2 patients, for one is a chronic alcoholic, and the other has chronic recurrent pancreatitis. One patient developed a thrombophlebitis at the site of an I.V. tolbutamide injection. Several patients have had transient mid-morning complaints suggestive of hypoglycemia. Blood specimens taken at the time of complaint have failed to reveal hypoglycemia, except in one instance. In the latter patient, when the dosage of tolbutamide was reduced, the complaints disappeared.

There have been several complications that have not been attributed to the tolbutamide therapy. Included among these are two deaths, one from uremia secondary to diabetic nephropathy, and the second from metastatic carcinoma to the liver. Three patients in the older age group have developed cerebral thromboses, and two have shown progression of retinopathy.

Several thousand laboratory determinations have been performed in a search for evidences of hepatic, renal, or hematological dysfunction resulting from the oral therapy. These include bromsulphalein retention, total and fractional serum proteins, cephalin flocculation, alkaline phosphatase, prothrombin time, cholesterol, amylase, blood urea nitrogen, complete blood count, platelet count, and urinalysis. To date, no evidence has been obtained of hepatic, hematological, or renal dysfunction that may be attributed to the tolbutamide therapy.

Summary

Seventy-five adult diabetic patients on tolbutamide therapy have been followed closely for periods up to one year. In 14 of the patients (19 per cent) tolbutamide therapy has been ineffective. An additional 3 patients have been lost to follow-up. The remaining 58 patients (approximately 80 per cent) have shown either a good or fair response and are still under observation. The previous diabetic state appears to correlate most closely with the results of tolbutamide therapy; that is, unstable diabetics or those difficult to control responded poorly, whereas stable diabetics responded well. Only minor toxic manifestations have been noted to date.

USE OF THE SULFONYLUREAS IN DIABETES MELLITUS*

By Samuel J. N. Sugar
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In a preliminary report[1] on management of diabetes with tolbutamide (Orinase†), successful control was noted in 76 per cent of 38 patients studied.

TABLE 1
ANALYSIS OF 143 CASES TREATED WITH TOLBUTAMIDE

Age	No treated	No successful	%
0–19	4	0	0
20–39	12	6	50
40–59	64	53	83
60–80	63	50	79
Duration of diabetes			
0–4 yr.	75	62	83
5–9 yr.	34	27	80
10–14 yr.	20	13	65
15–25 yr.	14	7	50
Insulin dosage			
0–19 units	57	46	81
20–39 units	56	42	75
40–59 units	15	11	73
60–85 units	15	10	66
Totals	143	109	76

Encouraged by these results, we made further observations in 143 cases. A few of these latter patients were given carbutamide for comparison of action.

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* The work reported in this paper was done in the Medical Department of Prince George's General Hospital, Cheverly, Md., and the Diabetic Clinic, District of Columbia General Hospital, George Washington University Division, Washington, D C

† The Orinase used in this study was supplied through the courtesy of C. J. O'Donovan of The Upjohn Company, Kalamazoo, Mich.

Method of Study

In patients controlled on insulin, tolbutamide was started 24 hours after the last dose of insulin. An initial "priming" dose of 2.5 gm. was followed in 24 hours by 1.5 gm. A maintenance dose of 1 gm. was given every morning thereafter. If control was difficult, the dose was increased. In some cases the daily dose was decreased as control improved. In hospitalized patients, blood sugars before and after breakfast, as well as 24-hour quantitative urine glucose, were estimated daily.[2] In addition, qualitative tests for sugar and acetone in the urine were made before meals and at bedtime. Diet was maintained at optimal caloric requirements.

In the outpatient clinics, those diabetics controlled with less than 20 units of insulin were started on tolbutamide 24 hours after the last dose of insulin. When more than this amount of insulin was being used, reduction in dosage was done in a gradual manner according to the effectiveness of tolbutamide.

All patients were advised as to the need for frequent testing of urine for sugar and acetone. On return to the clinic, the records of these tests, as well

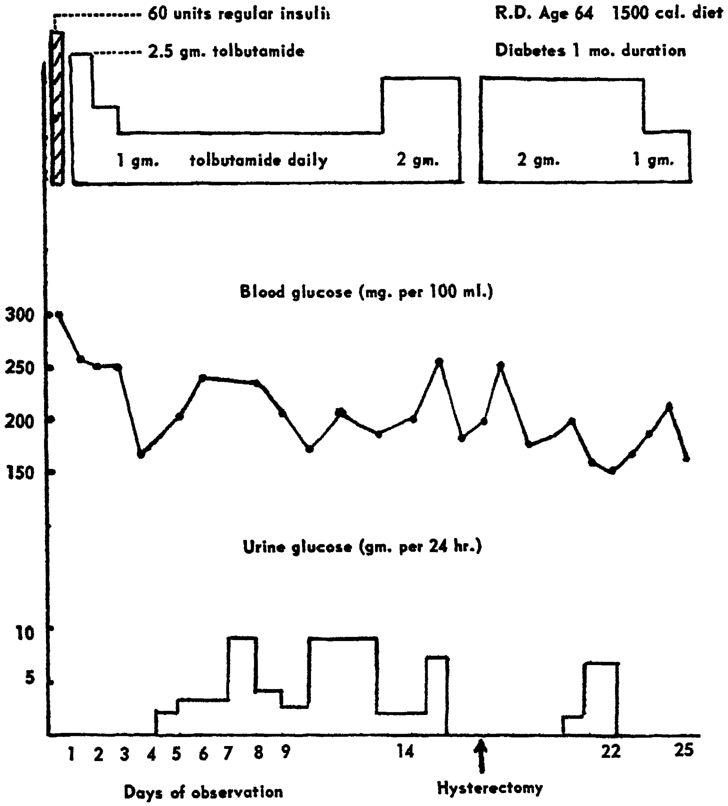

FIGURE 1. Good control with tolbutamide before and after major surgery.

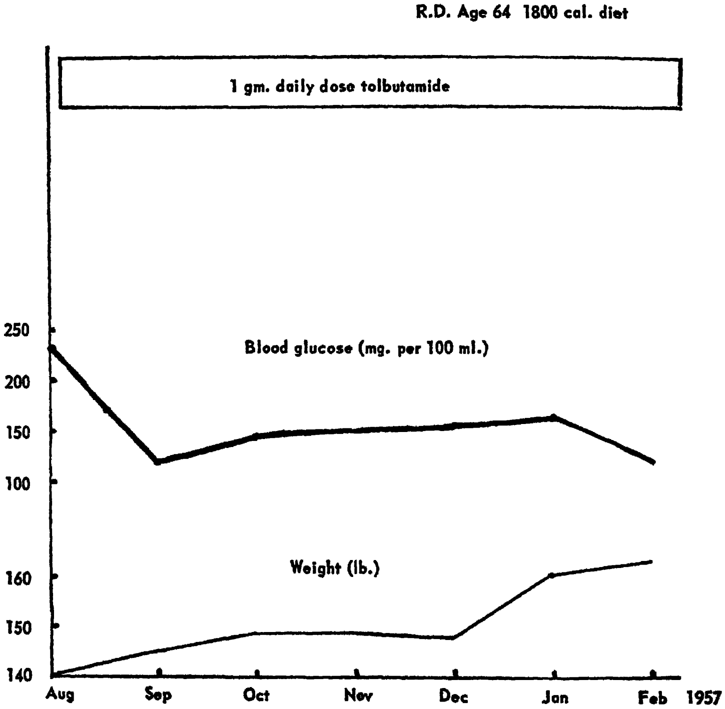

FIGURE 2. Good control with tolbutamide continuing after surgery. Gradual weight gain.

as of that day's blood sugar, were studied to obtain an estimate of the degree of control. Management with tolbutamide was considered satisfactory if the average blood sugar was less than 200 mg. per 100 ml. of blood, and if the daily excretion of glucose in the urine was kept below 15 gm.

Results

An analysis of 143 cases treated with tolbutamide is presented in TABLE 1. One hundred and nine patients (76 per cent) studied for more than 2 months showed adequate control, as measured by the criteria mentioned above. Of the 34 failures (24 per cent), 10 were "juvenile" type diabetics, requiring large doses of insulin and with a pronounced tendency to acidosis. For such reasons as fear of injections, allergy, or recentness of onset of diabetes, 23 patients had not previously taken insulin. These cases were quite easily controlled with tolbutamide. Two patients underwent major surgery while taking the drug. One of these (FIGURE 1) had panhysterectomy and radical node resection for carcinoma of the cervix. Tolbutamide was omitted on the day of operation and resumed in 24 hours. Diabetic control has remained good for 6 months (FIGURE 2). Several patients with ketosis were given a trial with tolbutamide. Only one of these (FIGURE

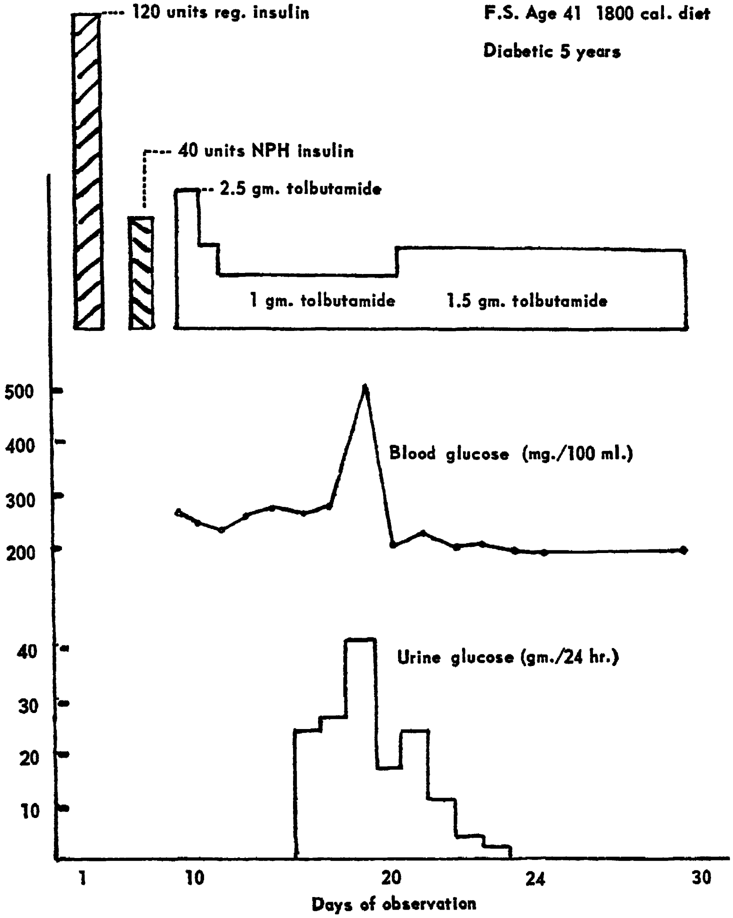

FIGURE 3. Patient relieved of ketosis with insulin, and satisfactorily controlled thereafter with tolbutamide.

3) responded. Acidosis was relieved by 120 units of regular insulin in 24 hours. Thereafter control was maintained with 40 units and, later, 1.5 gm. of tolbutamide daily in divided doses, 2 tablets before breakfast and 1 before supper. One case of chronic ulcerative colitis and diabetes improved remarkably with carbutamide at a dose of 1 gm. twice daily. After 2 months tolbutamide was substituted for the carbutamide. No exacerbations of the colitis have been noted after 9 months of therapy. Three patients have been able to stop medication and yet remain well controlled for as long as 3 months. One of these (FIGURE 4) has "never felt better."

One young mother became pregnant soon after diabetes was discovered. Increasing hyperglycemia and glycosuria were not satisfactorily managed by diet alone. After the usual starting dose, administration of 1 tablet of tol-

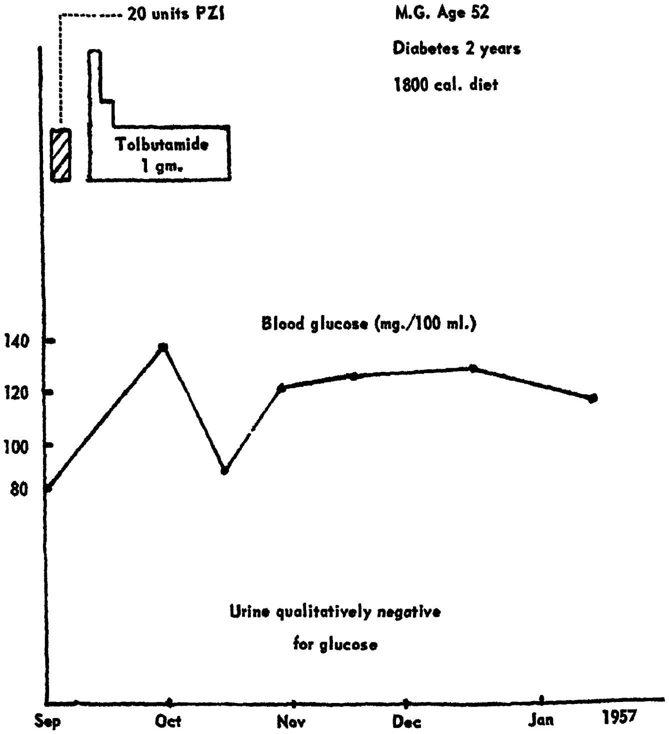

FIGURE 4. Good control continuing after stopping of tolbutamide.

butamide (0.5 gm.) daily was sufficient to control the diabetes for the entire pregnancy (FIGURE 5). The patient was delivered by Caesarean section in her 36th week and has remained in good control for 2 months. The infant boy (11 lb.) had a normal blood sugar and no glycosuria. He is developing normally.

Toxicity

As a rule, side reactions to tolbutamide were not too severe. In a third of the patients there was a prominent tendency to gain weight (TABLE 2). One case of leukopenia with a white blood count of 1800 was noted. This was not permanent, and tolbutamide was resumed without ill effect. Three patients went into congestive failure while on the drug. One of these had previously had pulmonary edema. All responded well to digitalis and mercurials.

One patient whose diabetes was complicated by chronic pyelonephritis and retinitis was given tolbutamide with good effect (FIGURE 6). However, after several months generalized itching followed by a purpuric rash on the legs (FIGURE 7) was noted. This increased in severity and was accompanied by

TABLE 2
SIDE REACTIONS TO TOLBUTAMIDE

	No. of patients*
Rash and itching	6
Intercostal neuralgia	2
Nausea and vomiting	3
Leukopenia (1800)	1
Hypoglycemic symptoms (anxiety, hunger, sweating)	7
Weight gain (1 to 4 lb.)	48

* Of a total number of 143 patients treated.

nausea and vomiting, so that the compound was necessarily discontinued. There was no unusual effect on the blood or urine. Hemoglobin was 13 gm. (Haden-Hauser), the white count 10,150. Only 10 units of lente insulin are needed at present. The rash has persisted for 2 weeks since cessation of tolbutamide but seems to be fading gradually.

Three deaths occurred in patients who were taking tolbutamide. One was

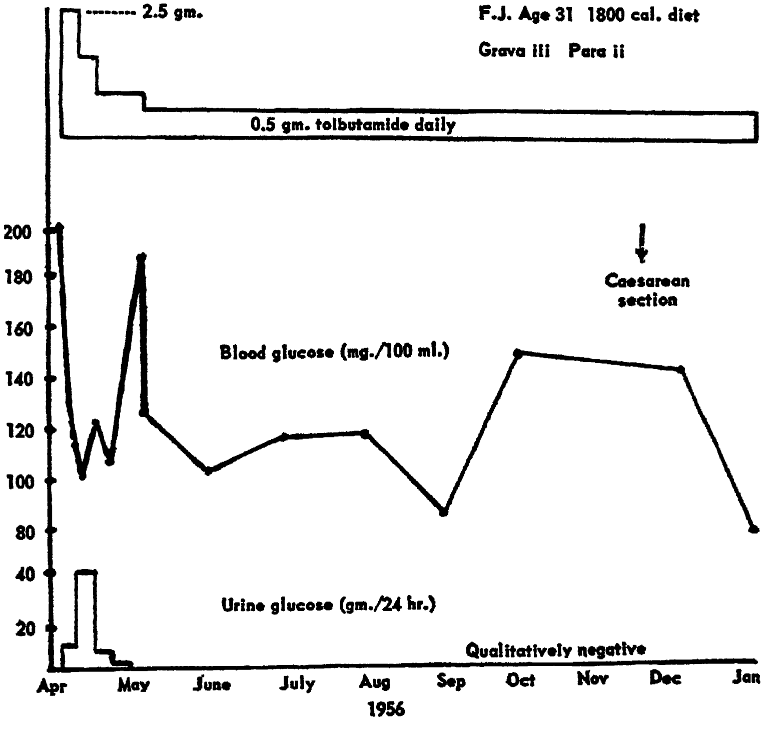

FIGURE 5. Good control during pregnancy and post partum.

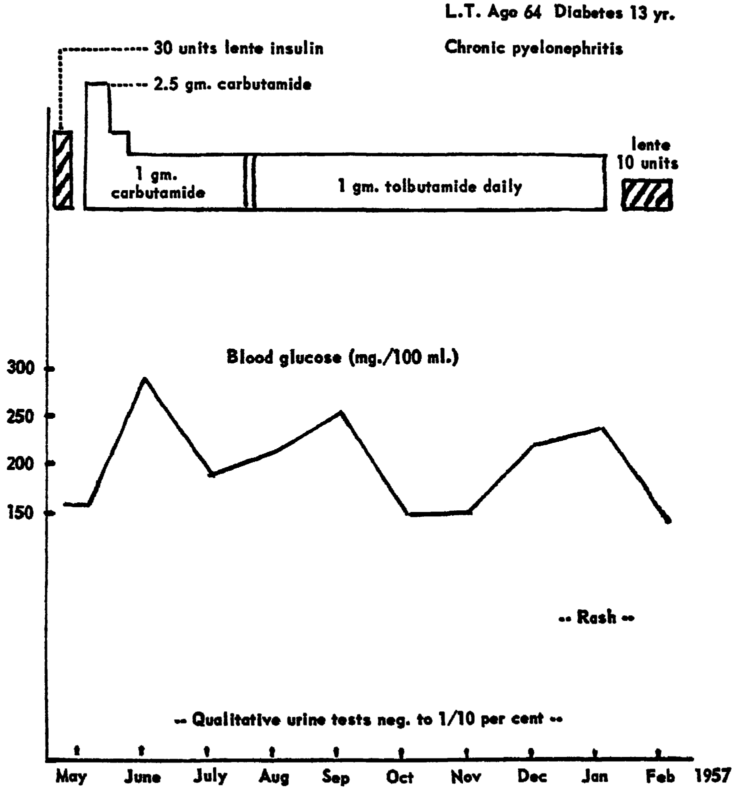

FIGURE 6. Fair control with sulfonylureas; severe itching and rash persisting after discontinuation of the drug.

due to generalized carcinomatosis, the second to overwhelming infection after midthigh amputation, and the third to hemorrhagic pancreatitis with fat necrosis following surgery for common-duct obstruction. Autopsy was obtained in the last case. Microscopic examination of the liver, kidneys, and pancreatic islets showed no effect attributable to tolbutamide.

Conclusions

(1) Of 143 diabetics studied, 76 per cent were satisfactorily controlled with tolbutamide.

(2) The most easily controlled were middle-aged patients with relatively recent onset of diabetes, requiring less than 40 units of insulin for control.

(3) Tolbutamide can be used on an ambulatory basis. Frequent urine testing and gradual reduction of insulin dosage while tolbutamide was administered gave optimal control.

(4) Unstable diabetics of any age are poor candidates for control with tolbutamide.

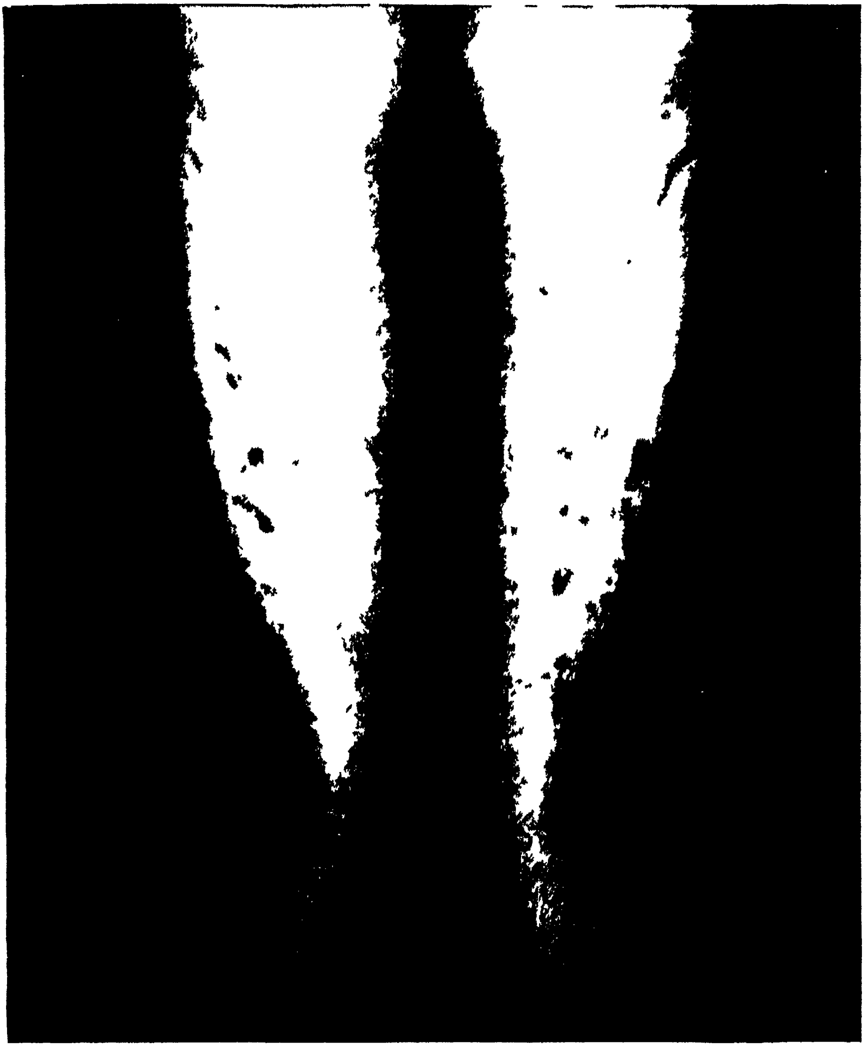

FIGURE 7. Purpuric rash on legs following administration of tolbutamide.

(5) Toxicity to tolbutamide was low. There was a prominent tendency to gain weight. Skin rash and itching could become severe enough to warrant discontinuance of the medication.

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FURTHER EXPERIENCE WITH THE USE OF SULFONYLUREAS IN DIABETES

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The continued use of tolbutamide (Orinase*) in human diabetes for 10 months has provided added information about its action. The group studied consisted of 90 ambulatory private patients (all adults, except 2 juveniles) under the close supervision of a single observer. All but 6 patients proved to be reliable in following their diet. This is vital for the validity of ambulatory studies.

As a result of this expanded experience, the blood sugar response test with tolbutamide for case selection[1] was abandoned. Instead, only clinical criteria were employed to decide whether to treat with tolbutamide and whether to replace insulin wholly or in part in a given patient. Moreover, a uniform dose of 2.0 gm. of tolbutamide per day was found to be practical as a starting dose for all patients. The blood sugar level 1 hr. after breakfast or lunch was used as an index of therapeutic accomplishment. The aim of treatment with tolbutamide was a blood sugar value of 140 to 160 mg. per cent (Folin-Wu), as in the case of insulin therapy. This rigid requirement was responsible for the relatively high average daily dose of tolbutamide in this group, namely, 2.5 gm.

In the initial regulation the probability of success could be gauged by the end of the first week, but the final dose required 3 to 4 weeks for stabilization. In the patients requiring higher doses, the blood sugar level (1 hr. after a meal) sometimes remained at a plateau of about 200 to 300 mg. per cent over the period of several weeks required to reach the effective dose of 3 to 6 gm. per day. It was only at that point that dosage dependency would be observed in such patients for the first time, and even 1 tablet per day would then exert a distinct effect.

The constancy of action of tolbutamide in those patients of this group with a longer period of regulation was then studied. Of the 90 patients, 35 who had been reliable in their diet (and weight) and had had over 100 days of therapy were chosen. Of these, 14 had been treated for over 200 days, and 6 for over 250 days An analysis was made of the variations in dosage between the end of the initial regulation (3 to 6 weeks) and the end of the study (up to 100 to 270 days). A change in dosage of 0.5 gm. per day of tolbutamide was considered significant. TABLE 1 shows the results: of the 35 patients, 22 showed no change, 8 showed a decrease, and 4 an increase in tolbutamide requirement. The one long-term juvenile patient showed irregular variations in dose that could not be classified. In the remainder, the changes noted occurred gradually and in one direction. Thus, the stability of the

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* The Upjohn Company, Kalamazoo, Mich.

TABLE 1
ALTERATION IN DOSE OF TOLBUTAMIDE DURING PROLONGED TREATMENT OF HUMAN DIABETES

Patients	Direction of alteration of dose of tolbutamide	Amount of change in dose of tolbutamide (grams per day)
Number		
22	Unchanged	
4	Increased	1, 1.5, 2 (2 patients)
8	Decreased	1, 0.5 (7 patients)
1	Variable	0 to 1.5, variable
35		

tolbutamide requirement in the therapy paralleled that of insulin. As a result, the patients required similar frequency and type of clinical observation.

At present, one of the recognized mechanisms by which the sulfonylureas exert their hypoglycemic effect is by stimulation of the pancreatic islets, both physiologically[2, 3] and histologically.[2, 4] This has raised the possibility of deterioration of the islets as a result of such stress. Accordingly, the clinical data have been examined for evidence of this, both in long- and short-term cases.

In this group there were 18 patients in whom the drug was unsuccessful and in whom insulin treatment was reinstituted after a short trial of sulfonylurea. Among most of these it was possible to appraise the stability of the diabetes under the influence of this therapy by using as an index the insulin requirement before and after therapy. Only 1 patient did show a striking change. This was a 50-year-old male with diabetes of 12 years' duration, who had been on insulin therapy for 3 years. He was well controlled on 16 units of NPH insulin and was reliable in diet. After 40 days of tolbutamide in increasing doses of from 2.0 to 4.5 gm., the postprandial blood sugar was still 265 mg. per cent (Folin-Wu), and this therapy was abandoned. On reregulation, the insulin requirement was 40 units NPH. This exceptional case was highly suggestive of a deterioration of carbohydrate metabolism or pancreatic islet function under tolbutamide therapy. Although such changes occasionally do occur spontaneously during insulin treatment of human diabetes, they are usually accompanied by a complication.

On the other hand, the long-term cases ordinarily afforded tolbutamide requirement as the only measure of carbohydrate metabolic efficiency. However, this is probably not a specific index of the integrity of pancreatic islets, since the sulfonylureas probably decrease blood sugar by extrapancreatic action also.[5] A simple and direct measure of pancreatic islet function is not available under these circumstances, but insulin requirement probably would be a closer approximation to this than the dose of tolbutamide. In the literature there are only isolated cases of reregulation of patients by insulin after cessation of sulfonylurea therapy.[6]

TABLE 2

EFFECT OF TOLBUTAMIDE ON THE REQUIREMENT OF EXOGENOUS INSULIN IN HUMAN DIABETES

No	Name	Age (years)	Duration of diabetes (years)	Duration of insulin (years)	Original insulin (units)	Tolbutamide therapy			Insulin reregulation	
						Final dose (grams)	Duration (days)	Dose change (grams)	Duration (days)	Change in needs (units)
1	D. R	62	5	5	22	2.5	188	0	11	0
2	A. F	49	1.5	1.5	20	1.0	224	Decrease 0.5	13	0
3	P. R.	54	14	1	8	2.0	248	Decrease 0.5	11	Decreased by 4
4	A. Y	64	4	4	32	2.0	262	0	8	0
5	S. C.	55	5	5	34	4.5	266	0	12	0
6	R. B.	53	1	1	18	0.5	224	Decrease 0.5	8	0
7	A. K.	54	9	2	16	4.0	275	Increase 1.0	10	0
8	P. C.	60	14	6	40	4.0	283	Increase 1.0	10	0

In an attempt to secure such information, 8 uncomplicated, long-term (over 6 months each) tolbutamide-treated diabetics were taken off the drug and reregulated with insulin. In none was the insulin requirement altered, as compared with that prior to the period of therapy (TABLE 2). Moreover, this stability of insulin requirement did not correlate with alterations in the dose of tolbutamide in 5 of these patients (Nos. 2, 3, 6, 7, and 8). If this is a valid index of pancreatic islet function, then it may be concluded that such function had not changed in these 8 patients after 6 to 9 months of therapy with tolbutamide.

In this series there were 2 juvenile diabetics successfully treated with tolbutamide. One was 14 and the other 13 years of age. The former, already reported,[7] had a variable tolbutamide requirement, and required omission of the drug for several days at a time due to hypoglycemic reactions. Very few instances of successful treatment of juveniles with these drugs have been reported.[8-10] Effectiveness in the 2 cases described here is probably best correlated with the recent onset of the diabetes in each (5 months) as well as the lack of development of ketonuria and only a moderate rise in hyperglycemia after the omission of insulin. The tolbutamide requirement was different in each, 0 to 1.25 gm. per day in the first, and 6 gm. in the second patient. This paralleled the insulin requirement, which was 4 to 8 units in the former and 22 units in the latter.*

Two cases in this group represented diabetes that first became manifest in the course of carcinoma of the large bowel with metastases. In neither case did the metastases affect a substantial portion of the liver. Such increase in severity of a diabetic condition, latent or manifest, by complicating disease is common, and the mechanism is not always clear. Tolbutamide, at an average dosage level (2 to 2.5 gm. per day), was rapidly effective in each patient.

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* Further details of these cases will be published separately.

Gastrointestinal symptoms have been noted during the use of tolbutamide.[7] They consist of heartburn, upper abdominal discomfort, or bloating to a significant degree, and they occur particularly in those patients with a previous history of such symptoms. It may occur in a patient with a gastrointestinal tract shown to be normal by X-ray examination. One patient of this series with known duodenal ulcer had a definite exacerbation following the use of tolbutamide. One other patient, a 75-yr.-old male, with no previous history of gastrointestinal disease and without warning symptoms, suffered sudden perforation of a duodenal ulcer. This occurred after 135 days of tolbutamide therapy with a dose of 2.5 gm. per day. These observations suggest the need for caution in the use of these drugs in the presence of a history of gastroduodenal symptoms. The precautionary use of antacids would be at least indicated under such circumstances.

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THYROID FUNCTION OF DIABETIC PATIENTS AS INFLUENCED BY THE SULFONYLUREAS

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Discovery of the hypoglycemic action of two sulfonylureas, carbutamide and tolbutamide, has been followed by their widespread trial in the management of diabetes mellitus. Their sulfonamide structure has led to a study of their influence upon the thyroid gland.

Goiters have been discovered in small laboratory animals subsequent to the feeding of carbutamide.[1-3] Some workers have demonstrated similar changes following the feeding of tolbutamide,[4] but others have been unable to confirm this.[2, 3]

As measured by the uptake of I^{131}, thyroid function in human beings has been decreased by proper dosages of carbutamide,[5-10] but no satisfactory clinical or laboratory evidence of a hypothyroid state has been demonstrated,[5, 7-10] although serum protein-bound iodine levels have occasionally been lowered.[6, 9, 10]

Most observers have failed to show any thyroid-depressant action for tolbutamide in commonly employed dosages; that is, up to 2 gm. daily.[5, 7-11] However, a slight lowering of thyroid function in man by tolbutamide has been noted by some observers.[5, 6, 12] This is a very weak action, and it is not well demonstrated unless relatively large doses, for example, 100 mg./kg. of body weight, are used.[12]

In view of the sulfonamide structure and the long-term usage implied in the application of these hypoglycemic agents to the treatment of diabetes mellitus, it has seemed advisable to study their influence on the function of the thyroid for relatively long periods of time.

In conjunction with the administration of these sulfonylureas, we have previously reported data from 55 older patients with mild diabetes subjected to repeated tests of thyroidal function over periods of time up to 20 weeks of observation.[8, 9, 10] Sixteen of these patients were studied only in short-term experiments concerned with the uptake of I^{131} by the thyroid. The results of this study may be summarized briefly:

(1) Following a single dose of 4 gm., both carbutamide and tolbutamide may depress the gradient of uptake of I^{131} by the thyroid; this action was striking with carbutamide and slight with tolbutamide.

(2) When 4 gm. of tolbutamide was given daily for 4 days, the uptake of I^{131} by the thyroid changed, but not significantly, from an average of 19.3 to an average of 16.5 per cent. In 4-gm. doses daily for 4 days, carbutamide reduced the I^{131} uptake of the thyroid from 23.3 to 5.3 per cent, a highly significant reduction.

(3) When 3 gm. of carbutamide was given daily for 4 days, the I^{131} uptake

of the thyroid dropped from 19.3 to 10.3 per cent, again a significant change, with a P value of less than 0.05.

In view of the above, the remaining 39 patients have been observed for periods up to approximately one year, and during this period the thyroid function of each patient was checked periodically; the present report deals with the results obtained.

Subjects and Procedures

The methods for selection of the 39 subjects discussed in this report have already been fully described.[10] Of these subjects, 18 were men with a range of age from 57 to 89 years and an average age of 69.7 years, while the remaining 21 were women whose ages ranged from 57 to 83 years and averaged 72.6 years. Of the patients originally receiving tolbutamide, 10 were men whose ages ranged from 59 to 89 years and averaged 71.8 years, and 16 were women whose ages ranged from 57 to 85 years and averaged 75.2 years. Of those originally receiving carbutamide, there were 8 men whose ages ranged from 57 to 76 years with an average of 67.1 years, and 5 women whose ages ranged from 65 to 81 years with an average of 74.2 years. Later, 9 subjects (5 men and 4 women) who initially took carbutamide were transferred to treatment with tolbutamide, and 7 individuals (3 men and 4 women) who at first used tolbutamide were later given carbutamide. Therefore, the over-all results of long-term therapy with these two sulfonylureas involve 35 trials with tolbutamide and 20 trials with carbutamide.

These patients had been admitted to the hospital originally for control of their diabetes or for some degenerative vascular disease. Fluctuations due to acute episodes in the course of either of these types of disease did not occur, in the patients here reported, during the period of observation covered by the treatment with the sulfonylureas.

The general condition of the patients, basal metabolic rates, serum protein-bound iodine determinations, and I^{131} uptakes by the thyroid were used as criteria for determining thyroid function. We used the Benedict-Roth metabolimeter, with commonly employed standards, for the performance of the basal metabolic rates. The method of O'Neal and Simms[13] was employed for the determinations of serum protein-bound iodine. Radioiodine uptake by the thyroid was measured by direct count with a Geiger-Mueller tube and by determining urinary excretion in a scintillation well counter. No single tracer dose of I^{131} exceeded 25 μc. and most of them were under this amount. In the year of observation no single subject received a total of more than 100 μc. of this material.

Results

From the standpoint of clinical findings, it may be said that at no time did any of the subjects show any bona fide evidence of hypothyroidism from the use of either drug in daily doses of 1 or 2 gm. over periods up to 47 weeks of treatment.

Basal metabolic rate. No significant changes occurred in the basal metabolic rates of 7 of the 10 subjects receiving carbutamide or in 9 of the 15 subjects receiving tolbutamide at the 1 gm. level of dosage over periods of time up to 43 weeks. Earlier data on these patients have been reported.[8-10] Since no essential changes took place in these first weeks of treatment or in the later observations, further details are not recorded. In the remaining patients, it was impossible to obtain reliable tests.

When 2 gm. of carbutamide daily was employed, the averaged pretreatment basal metabolic rate was +4.3 per cent, that at 9 weeks 1.6 per cent, and that at 43 weeks 7.9 per cent, with variations that make none of these figures statistically significant. However, 2 of the 8 subjects on which it was possible to carry out the tests had values below −10 per cent at the ninth week, with values within the normal range in the control periods and at the end of the therapeutic period (19 to 22 weeks).

When 2 gm. of tolbutamide was administered daily, the averaged pretreatment basal metabolic rate in 14 of the 20 patients on whom the determination was carried out was +0.9 per cent, that at 9 weeks +2.3 per cent, and that between 43 and 47 weeks +7.9 per cent. There were wide fluctuations, all within the normal range, so that the above differences have no significance.

Serum protein-bound iodine. With neither carbutamide nor tolbutamide in daily doses of 1.0 gm. for periods of time up to 22 weeks were there any striking variations in the serum protein-bound iodine, and all values stayed within the normal range. The same statement holds true for tolbutamide in 2-gm. doses over periods of time up to 47 weeks. Data were obtained from 6 subjects who received carbutamide in daily 2-gm. doses. In these the control values ranged from 4.0 to 7.5 μg. per 100 cc., with an average of 6.1. (Normal limits of the method in our hands were 4.0 to 8.0 μg. per 100 cc.) At 9 weeks the range was from 3.0 to 5.2 μg. per 100 cc., with an average value of 4.4. In only one subject was the posttreatment value depressed beyond the accepted "low-normal" figure. Unfortunately, no determinations were carried out at a later time. The differences obtained have no statistical significance.

When 2 gm. of tolbutamide was used daily for 43 weeks, the averaged value for serum protein-bound iodine decreased from 5.8 to 4.8 μg. per

Table 1
The Influence of Tolbutamide (2 Gm. Daily) on the Serum Protein-bound Iodine of 14 Diabetic Patients

Period (weeks)	Serum value (μg. per 100 cc.)	
	Range	Average
Control	3.9–7.7	5.8
17	4.0–7.6	5.1
43	2.3*–7.1	4.8*

* Two values were less than 4.0 μg. per 100 cc.

100 cc.; the range before treatment was 3.9 to 7.7 and after treatment 2.3 to 7.1 μg. per 100 cc. (TABLE 1). From TABLE 1 it can be seen that when the drug was administered over periods of time up to 43 weeks, changes in serum protein-bound iodine were slight and not statistically significant.

Radioactive iodine (I^{131}) *uptake by the thyroid.* We have already mentioned the results of 4-gm. doses of carbutamide given singly and daily for 4 days; that is, a statistically significant depression of I^{131} uptake by carbutamide and a slight but not significant depression of such function by tolbutamide.[8-10] In doses of 1 gm. daily for 17 and 20 weeks respectively, neither tolbutamide (in 15 patients) nor carbutamide (in 10 patients) appreciably influenced the uptake of I^{131}.

TABLE 2
INFLUENCE OF DAILY DOSES OF 1 GM. OF CARBUTAMIDE AND TOLBUTAMIDE ON I^{131} UPTAKE OF THE HUMAN THYROID GLAND

Period (Weeks of therapy)	Percentage uptake			
	Carbutamide (10 subjects)		Tolbutamide (15 subjects)	
	Range	Average	Range	Average
"Fore period"	11–38	23.0	11–36	21.4
2–3	12–36	23.0	10–28	18.9
8–9	12–24	20.2		
10			5–27	16.9
17			16–23	20.2
19–20	12–28	21.8		

TABLE 3
EFFECT OF TOLBUTAMIDE (2 GM. DAILY) ON THYROIDAL UPTAKE OF I^{131} BY DIABETIC PATIENTS

Period in weeks (No. of subjects)	I^{131} Uptake (%)	
	Range	Average
Control (20)	8–37	20.3*
2–3 (20)	7–47	19.4
5–6 (3)	13–20	16.7
9 (15)	4–35	16.9
20–22 (16)	14–40	24.8
43–47 (7)	19–36	24.0

* S.D. = ±4.3

TABLE 4

EFFECT OF CARBUTAMIDE (2 GM DAILY) ON THYROIDAL UPTAKE OF I^{131} IN DIABETIC PATIENTS

Period (weeks)	No subj	Per cent uptake (24 hr)	
		Range	Average
Control	10	10–31	20.0
2	3	14–20	16.3 } 10.5
3	7	0.5–11	4.1
5	3	5–13	10.9
9	10	6–20	11.2
19–22	4	20–44	32.0

The values for these subjects are shown in TABLE 2. The average "fore period" and 17-week values for tolbutamide were 21.4 and 20.2 per cent, respectively, and those for carbutamide in the "fore" and 19- to 20-week periods were 23.0 and 21.8 per cent, respectively. None of these differences is significant, nor indeed were any of the intermediate figures—not even the drop to 16.9 per cent with tolbutamide at the tenth week, as the S.D. at this point was ±4.3 per cent (TABLE 2).

When 2 gm. of one of the sulfonylureas was used daily, the story was somewhat different (TABLES 3 and 4; FIGURES 1 and 2). In explanation of these tables and figures, it may be well to indicate that any value derived from less than the total number of cases concerned was referred to the average control

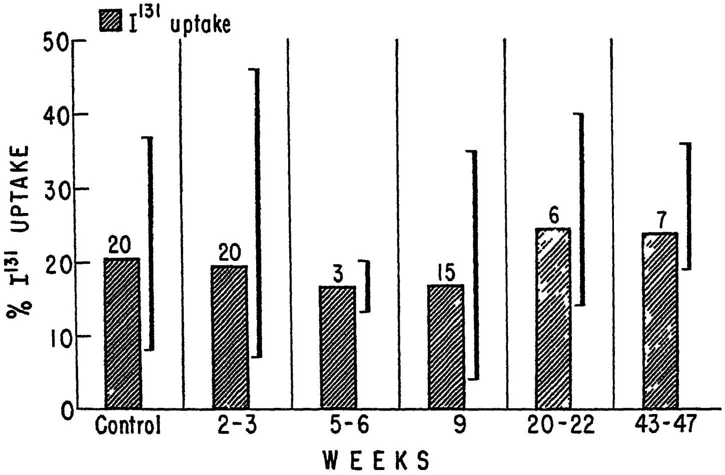

FIGURE 1. Effect of tolbutamide (2 gm. daily) on thyroidal I^{131} uptake. Vertical lines represent range of values. Numbers at the top of each bar represent number of subjects on whom determination was made.

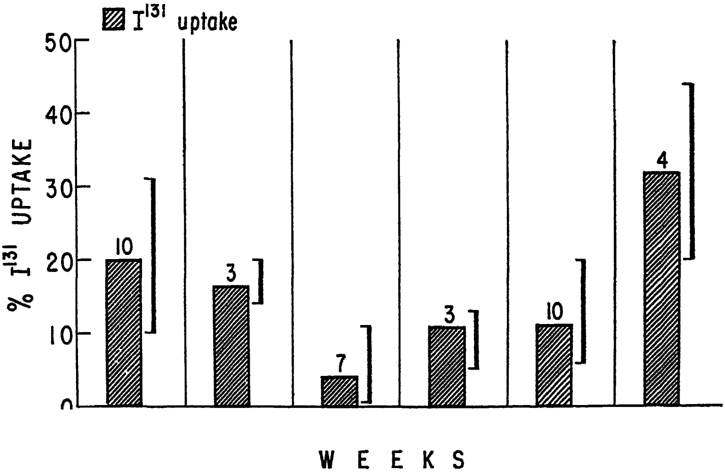

FIGURE 2. Effect of carbutamide (2 gm. daily) on thyroidal I^{131} uptake. (See legend of FIGURE 1 for explanation.)

value for those specific cases, and that a proportionate value was calculated if this control deviated more than ±2.5 per cent from the average control of the whole group (shown by an asterisk in the tables). With tolbutamide, the averaged value for I^{131} uptake at 6 and 9 weeks was below that of the control but, in view of a standard deviation of ±4.3 per cent for the method and of the wide individual fluctuations, these changes are not significant, nor is the increase above the control after 22 and 47 weeks of treatment, respectively (TABLE 3 and FIGURE 1).

When carbutamide, 2 gm. daily, was given, there was a highly significant depression of uptake at the third week (*P* value less than 0.01) and at 9 weeks (*P* value less than 0.05), as shown in TABLE 3 and FIGURE 2. The surprising thing about this study was that the radioiodine uptake was above the control value at the end of 22 weeks of carbutamide therapy.

Summary

(1) Thyroid function, as measured by the clinical condition of the patient, by the basal metabolic rate, by the value for serum protein-bound iodine, and by the uptake of I^{131} has been studied in 55 patients with diabetes mellitus while under treatment with carbutamide or tolbutamide.

(2) Sixteen patients were used solely for short-term experiments in which it was shown that carbutamide vigorously depressed the uptake of I^{131} for as much as 24 hr. after a single dose of 4 gm., while tolbutamide exerted little or no influence.

(3) No significant changes in basal metabolism or serum protein-bound iodine were produced by either drug in daily doses of 1 or 2 gm. over periods of time up to 47 weeks. However, at the end of 9 weeks, isolated cases treated with 2 gm. of carbutamide daily showed levels for serum protein-bound iodine and basal metabolism below the accepted range of normal.

(4) Radioactive iodine uptake by the thyroid was not appreciably disturbed by the administration of either 1 or 2 gm. of tolbutamide daily for periods of time up to 47 weeks.

(5) One gram daily of carbutamide did not influence the uptake of radioiodine by the thyroid.

(6) Two grams daily of carbutamide suppressed the uptake of radioiodine by the thyroid to approximately 20 per cent of its former value by the end of the third week of treatment. This was still depressed to 56 per cent of the control value at the end of the ninth week of treatment. However, radioiodine uptake and excretion were again normal after 19 to 22 weeks from the beginning of treatment.

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EXPERIENCE WITH THE TOLBUTAMIDE TREATMENT OF FIVE HUNDRED CASES OF DIABETES ON AN AMBULATORY BASIS

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Although my experience extends to more than 700 patients treated with tolbutamide (Orinase) for diabetes mellitus, this report is confined to 500 patients studied for periods of from 3 months to 1 year. All were private patients and represented the living and eating habits of ambulatory patients in whom a realistic test of the eventual clinical application of this drug could be evaluated. One hundred and fifty similar patients from the diabetic clinic of The Mount Sinai Hospital are not included in this report, but will be the subject of a subsequent paper by my collaborators, J. J. Bookman, R. Joelson, and G. Brill.

The sexes of the 500 patients were about equally divided; there were 260 males and 240 females. An index of the age category most suited for tolbutamide treatment may be gleaned from the fact that, of the 500 patients, 451 are now in the age bracket of 41 to 80 years, while only 38 are from 21 to 40 years old, and a meager 11 cases are now aged 20 or less (TABLE 1). In the latter group, I can report no single successful result. This breakdown is not to be confused with correlations as to the age at onset of diabetes. As 90 per cent of our patients are older than 40, a good or excellent response to tolbutamide treatment could be expected in 70 per cent of this group. From this one can deduce the broader extension of the possible therapeutic success with tolbutamide in the general population.

Correlation of Age at Onset of Diabetes With Duration

The purported factor of duration of diabetes influencing therapeutic success with tolbutamide is not substantiated in my experience. TABLE 2 shows the

TABLE 1
CORRELATION OF AGE AT TIME OF TREATMENT WITH RESPONSE TO TOLBUTAMIDE

Age at time of treatment	Total cases	Response to tolbutamide			
		Excellent	Good	Fair	Poor
0–20	11	0	0	1	10
21–40	38	10	5	4	19
41–80	451	242	117	18	74
Total.. .	500	252	122	23	103

TABLE 2
CORRELATION OF DURATION OF DIABETES WITH RESPONSE TO TOLBUTAMIDE

Duration	Total cases	Response to tolbutamide			
		Excellent	Good	Fair	Poor
<1	42	27	6	1	8
1–2 years	100	50	21	5	23
3–5 years	90	42	23	6	19
6–10 years	132	74	34	5	25
11–20 years	111	47	33	5	24
>20 years	25	12	5	1	7
Total	500	252	122	23	106

TABLE 3
CORRELATION OF DURATION OF DIABETES WITH RESPONSE TO TOLBUTAMIDE
WHERE THE AGE AT ONSET WAS 1 TO 20

Duration	Total	Excellent	Good	Fair	Poor
<1	6	0	0	0	6
1–2	3	0	0	0	3
3–5	1	0	0	0	1
6–10	6	0	1	1	4
11–20	2	1	0	0	1
>20	2	2	0	0	0
Total	20	3	1	1	15

response to treatment in relation to the duration of diabetes; it is evident that no significant correlation existed. On the other hand, in the juvenile diabetic (TABLE 3) such a correlation may be demonstrated in reverse, since even with the most recent onset of juvenile diabetes, the drug failed. Therefore, it is apparent that, regardless of the duration of diabetes, except in juvenile cases, tolbutamide treatment should not be withheld solely on the basis of duration.

In illustration, I cite the case of a 79-yr.-old man whose diabetes began in 1904. After two decades of starvation treatment, he graduated into the insulin era and has required 30 units of insulin daily for the past 30 years. He had constant glycosuria and hyperglycemia until one year ago when, with great reluctance on my part, he persuaded me to initiate tolbutamide treatment. Since then he has been singularly aglycosuric and normoglycemic with 1.5 gm. daily, a record never achieved with insulin.

Other Correlations

As to the reported influence of obesity on the response to tolbutamide: in this group of patients, 80 per cent were normal or underweight, and their

TABLE 4

CORRELATION OF RESPONSE TO TOLBUTAMIDE WITH RESPONSE ON NO THERAPY

Response to tolbutamide	Total cases	Response prior to use of tolbutamide			
		Excellent	Good	Fair	Poor
Excellent	41	0	0	6	35
Good	10	0	0	0	10
Fair	2	0	0	0	2
Poor	5	0	0	0	5
Total	58	0	0	6	52

response to treatment was as good as that of the 20 per cent who were obese. Moreover, I was not able to correlate the character of the onset of diabetes with the result of tolbutamide treatment. The onset was symptomatic in 60 per cent of the 500 patients, although the clinical result was good or excellent in two thirds of this group. To be sure, the patients with asymptomatic onset displayed almost uniform success with tolbutamide treatment, but this group was usually not treated with insulin.

As to the past history of acidosis and coma, a striking correlation could be deduced. In no instance was a patient with a past history of diabetic coma found to display a good response to tolbutamide. On the other hand, ketosis and/or acidosis did not prejudice the future response, since one third of the 34 patients with such a history subsequently exhibited a good clinical result with the drug.

In 58 patients, previously untreated diabetes, with or without ketonuria, proved an interesting challenge to the clinical effectiveness of tolbutamide (TABLE 4). Good and excellent responses were obtained in 51 patients, so that glycosuria, ketonuria, and classic symptoms were corrected in remarkably short periods.

The diet-treated patients with poor control comprised 131 of the total group of 500 patients. As expected, 121 of these served as excellent material to prove the effectiveness of tolbutamide in controlling diabetes. Only 10 patients failed to improve and subsequently required insulin. In the insulin-treated diabetic patients, success in replacing insulin by tolbutamide was possible in 202 of 315 patients. In addition to the qualitative change from insulin to tolbutamide, it was surprising to find quantitatively better diabetic control with the latter drug. The 113 failures were all restored finally to insulin treatment without tolbutamide and without loss of insulin tolerance. I do not feel that there is any benefit to be obtained from a combined therapeutic program of insulin and tolbutamide. After following the progress of 90 such patients for 1 year, I have come to believe that tolbutamide either replaces insulin or it does not—it cannot reduce the insulin dose significantly. There is no role for tolbutamide as partial treatment.

TABLE 5

CORRELATION OF PRIOR INSULIN DOSE WITH RESPONSE TO TOLBUTAMIDE

Prior insulin therapy	Total cases	Response to tolbutamide			
		Excellent	Good	Fair	Poor
<10 units	21	16	2	0	3
11–20 units	104	60	30	3	9
21–30 units	79	31	20	3	25
31–40 units	49	10	12	3	24
41–50 units	29	6	9	2	12
51–60 units	8	0	2	0	6
61–80 units	15	1	2	4	8
>80 units	12	1	2	1	8
Not on insulin	189	127	43	7	11
Total	500	252	122	23	106

Another purported deterrent to a successful result with tolbutamide has been the previous insulin dose required by patients. As illustrated in TABLE 5, the wide range of the size of the insulin dose between 10 to 100 units did not lend itself to such an interpretation. To be sure, the preponderance of the dosage hovered in the range of 20 to 40 units. Although successful treatment with tolbutamide was infrequent in those requiring more than 50 units daily, occasional striking success could be obtained even in a 100-unit case, and one should not be deterred from trying to replace insulin with tolbutamide because of the magnitude of the insulin requirement. As a corollary, no relation could be demonstrated between the duration of insulin treatment and the tolbutamide response; excellent results were obtained in one half of the patients who had been taking insulin for 20 years or more.

Side Reactions

Side reactions to tolbutamide treatment were limited to 4 skin reactions of a transient erythema that disappeared with continued therapy, 5 instances of marked face and body flushing after alcoholic beverages, and 2 cases reporting epigastric fullness. These side reactions could not be correlated with either the size of the dose or the duration of administration. To date, 28 patients have taken between 500 and 1000 gm. of tolbutamide. The daily dose usually averaged between 0.5 and 3 gm., with 60 per cent of the patients taking 1 gm. daily; 30 per cent, 3 gm. daily; and the remaining 10 per cent taking doses ranging between 0.5 and 3 gm. or more. Twenty-six patients were maintained on 3 to 6 gm. daily without untoward effects.

In the entire series there was no evidence in any single case of toxic reactions involving the hematopoietic, hepatic, renal, or cardiovascular systems. Tolbutamide was given to a number of patients with renal damage due to diabetic nephropathy, and showed no evidence of significant aggravation of the existing damage. Similarly, several patients who had recently recovered from hepatitis or obstructive jaundice were treated with tolbutamide without evidence of any resultant hepatic dysfunction.

Hypoglycemia, strictly speaking not a side reaction but a specific effect of tolbutamide, occurred in a dozen instances, but to a mild degree. There was never any clinical evidence of moderate or severe hypoglycemia. Tolbutamide-induced hypoglycemia proved easily amenable to oral administration of carbohydrate, with prompt response, and it was easily corrected by reduction of the dose.

There were two deaths among the 500 patients, and both were due to coronary thromboses in patients who had had several previous attacks of this disease. Neither death could be attributed directly to tolbutamide.

Summary

Five hundred diabetic patients were treated with tolbutamide over a period of 1 year. Ninety per cent of this group were 40 years of age or older, and 70 per cent of the patients in this whole group presented an excellent response to treatment with this drug. Included in the group were 315 patients who had been treated with insulin for years; in 202 of these tolbutamide replaced insulin completely. As was expected, there were striking therapeutic results in the non-insulin-treated patients, with excellent responses to tolbutamide in 90 per cent of this group. Except for the correlation of age at onset of diabetes, there appeared to be no influence in the eventual response to tolbutamide due to sex, body weight, insulin dose, duration of insulin therapy, or history of ketosis. The only two types of patient in whom therapy was unsuccessful were those who had experienced onset of the disease in childhood (juvenile, or under 20) and patients with a history of coma. No single serious or toxic effect was demonstrated in the entire group over a 1-year period.

REPORT ON EXPERIENCES IN ONE AND A HALF YEARS OF ORAL TREATMENT OF DIABETES WITH TOLBUTAMIDE

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For the last eighteen months we have been treating diabetes with tolbutamide (Orinase). During that time, 550 patients have been stabilized on tolbutamide in our clinic. Fifty of these patients received combined treatment of insulin plus tolbutamide. I shall not deal with the latter group in detail since observation of these cases did not demonstrate anything that has not already been published.[1, 2, 3]

Four hundred and fifty of our patients (FIGURE 1) are now being treated with tolbutamide. Of these, 410 are more than 50 years old, and most of them are more than 60. In 17 patients, the diabetes mellitus became manifest before they were 40 years old.

The stabilization on tolbutamide was carried out exclusively in the clinic, on the metabolism ward. One hundred and seventy patients had been previously treated with insulin; 133 patients were poorly controlled by diet alone; and 147 patients were newly detected diabetics who could not be controlled with diet alone. With all the patients of the last two groups, a strict dietetic regimen was tried first. Three carbohydrate-free test days were ordered, followed by a limitation of the carbohydrates to 120 gm. Only if, within 1 to 2 weeks, no fair balance was reached on a diet limited in

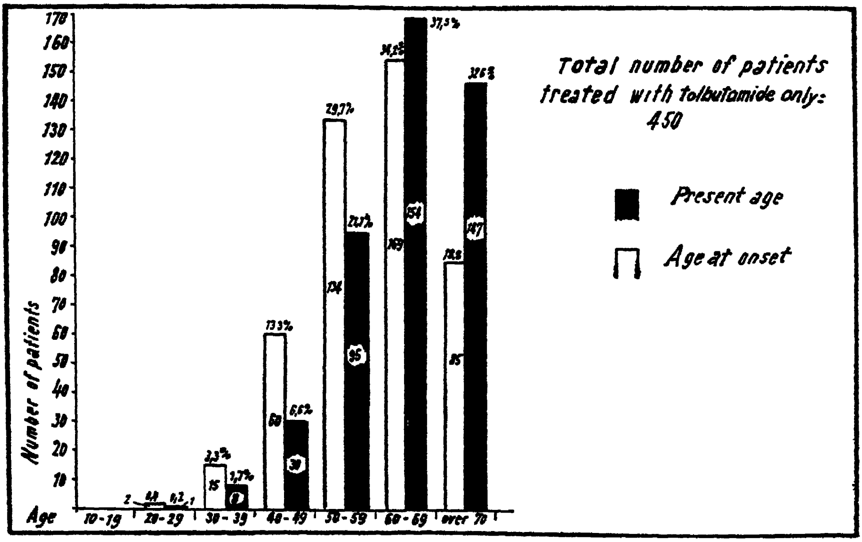

FIGURE 1. Age distribution of patients treated with tolbutamide only.

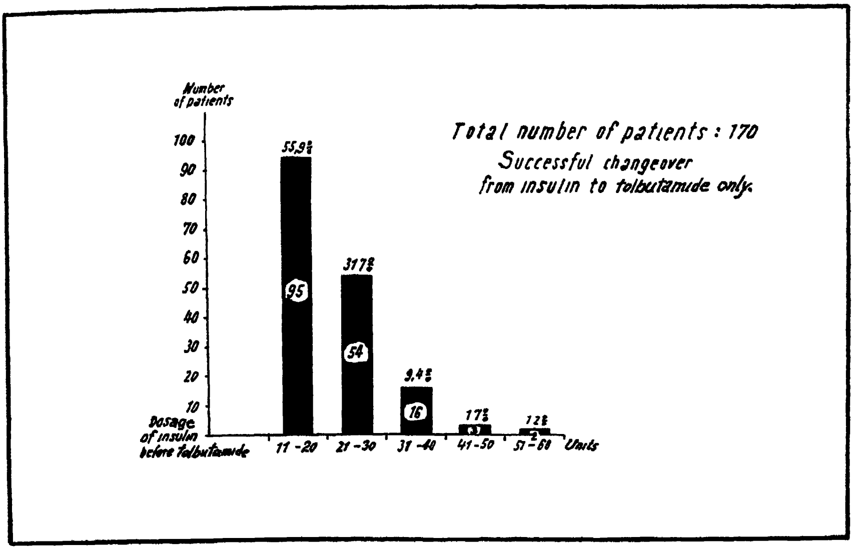

FIGURE 2. Successful transfer from insulin to tolbutamide.

carbohydrates was treatment with tolbutamide started. These two groups of patients undoubtedly include a number of patients who might have been treated with diet alone for some time.

With the 170 patients treated with insulin, the amount of insulin given was reduced until glycosuria and an increase of the blood sugar occurred. Then administration of insulin was stopped for 24 hours. If metabolic decompensation was observed, administration of tolbutamide was not attempted. It would seem noteworthy that even in several older patients with low insulin requirement—for instance 20 I.U. per day—we were able to observe within one day a metabolic decompensation, with blood sugar increases up to 600 mg. per cent and acidosis.

Among 170 patients (FIGURE 2), about 88 per cent required less than 30 I.U. of insulin.

In about 86 per cent of the cases (FIGURE 3), the duration of the insulin treatment was less than 5 years. However, 23 patients had already been receiving insulin for more than 10 years.

FIGURE 4 shows the daily amounts of tolbutamide that are required by our patients. For 400 patients the maintenance dose is 1.5 gm. or less per day. All these patients are in good metabolic compensation.

With some of the remaining 50 patients, the lowest maintenance dose of tolbutamide has not yet been reached, and some show only fair to poor stabilization. Among these, there are several patients who actually should be treated with insulin again. Some, however, refuse a return to insulin treatment and, in other cases, we have been content with a moderate metabolic stabilization because some of these patients were persons with cardial decompensation and others were patients suffering from frequent anginal

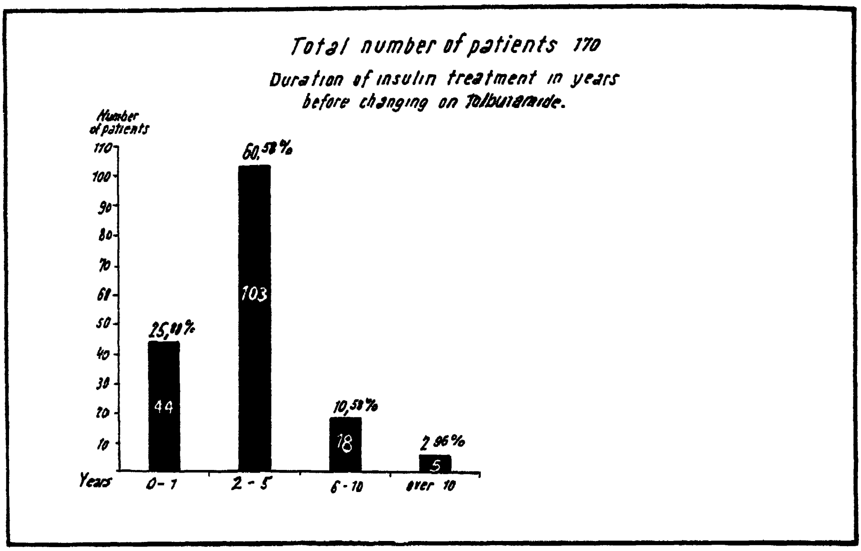

FIGURE 3. Duration of insulin therapy before changing to tolbutamide.

attacks where insulin therapy would hardly give better results or would perhaps even be connected with a greater risk. The metabolic compensation of all the other patients meets the requirements of a good stabilization.

Surprisingly few of our diabetics treated only with tolbutamide have experienced a transient decompensation. In most of these cases it was sufficient to admonish the patients to observe the diet strictly. In some cases it was necessary to administer small amounts of insulin in addition, for a brief period; usually, 20 I.U. of some depot preparation with a long-lasting action was sufficient, and often administration only every other day was adequate. In only 13 patients was it necessary to discontinue administration of tolbutamide completely and to readminister insulin. In general, the amounts of insulin required in this group were the same as before the treatment with tolbutamide. Four of these patients, who had been excellently stabilized on tolbutamide for a considerable time, experienced a decompensation for reasons which were not clear. Two of these had shown a slight acidosis during the 3 carbohydrate-free test days; they had been admitted to the hospital with severe acidosis. The other 4 patients, who had never been acidotic, suddenly showed severe glycosuria, the reasons for which were unknown. These patients, too, had to be changed over to insulin. So far, no attempts to return to treatment with tablets have been carried out. The stabilization on tolbutamide of the other 9 patients was fair to poor from the beginning, so that there had been doubts at once as to the success of a changeover. Thus, in all the patients, no substantial aggravation of the diabetes seems to have taken place during the last 1½ years.

Of all the patients who have received tolbutamide for periods ranging from 1 to 11 months, a total of 10 have died meanwhile. The average age was

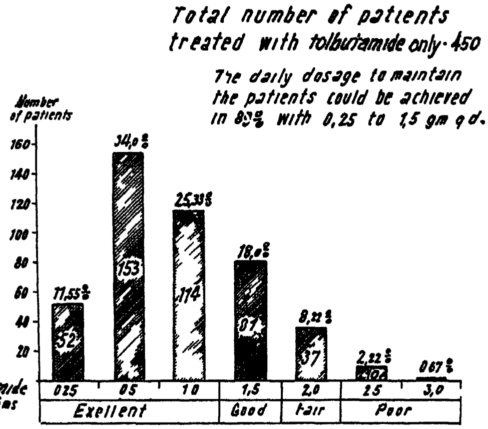

FIGURE 4 Daily dosage of tolbutamide required by patients.

73 years; the youngest patient in this group was 61 years old. This patient suffered from myocardial infarction before his diabetes was discovered. The average total dose administered to patients of this group was 200 gm. (ranging from 25 to 600 gm.). All patients showed sclerotic changes. Six patients died from heart failure (5 myocardial infarctions, 1 heart failure with decompensation). Three of them had chronic infections: 1 patient suffered from hypertrophy of the prostrate, chronic infection of the renal pathways, and chronic uremia; the second patient had gangrene and died from pulmonary embolism, the third patient (78 years old) suffered from a long-standing cholecystitis, enlargement of the liver, cholelithiasis, and cholangitis, with complicating pyelonephritis, and a final collapse of the liver functions, with jaundice. The tenth patient died from an acute infection (bilateral bronchopneumonia). None of these patients died either from diabetes or from allergic manifestations, or with other symptoms that might be associated with the tolbutamide treatment.

Ten additional patients are no longer under our ambulatory observation, so that nothing can be said about them as yet. When these patients left our control, most of them were transferred to insulin therapy in spite of their good stabilization, since we consider supervision by experts to be necessary during treatment with tolbutamide.

Dermatological reactions were observed in only 2 cases. These patients showed a slight urticarial exanthema; in 1 case, at the patient's request, it was possible to resume the treatment with tolbutamide after the skin eruption had disappeared. On the other hand, tolbutamide treatment was successfully started in 3 patients who had skin diseases prior to treatment. Under simultaneous treatment with hydrocortisone ointment and in connec-

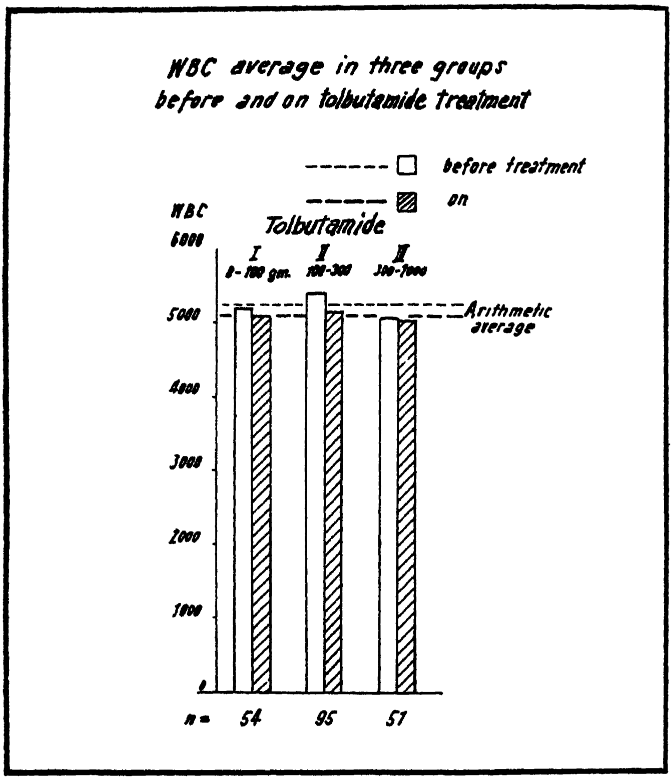

FIGURE 5. White blood cell count average in three groups before and during tolbutamide therapy.

tion with metabolic improvement, the dermatological symptoms largely receded.

Further side effects that were reported were incompatibility with the stomach and pressing sensations in the head; in the latter case, treatment with tolbutamide was abandoned. Also, 2 patients who said that they had felt better under the previous insulin treatment were returned to insulin although their metabolic compensation with tolbutamide had been good.

Controls of the blood picture were carried out on 200 patients (FIGURE 5) who had been under treatment with tolbutamide for a considerable time, and on 100 patients treated with insulin; this was done before and during treatment (TABLE 1). Computation of the above values, as well as preparation of a collective blood picture, before and during treatment, show no substantial deviation of the individual blood picture values. In particular, there is no significant decrease in the total leukocytes. We see that under insulin treatment the eosinophils, the band cells, and the neutrophils increase slightly so that the total of 68 per cent is 4 per cent higher than it was prior to treatment. Under insulin therapy, the lymphocytes decrease more than under tolbutamide, so that the total of white blood cells is lower than before

TABLE 1
THE EFFECTS OF TOLBUTAMIDE AND INSULIN ON THE BLOOD PICTURE

	Basophils	Eosinophils	Band cells	Neutrophils PMN	Total	Lymphocytes	Monocytes	Total	WBC	HGL gm. %
Before treatment (300 patients)										
Absolute	14.33	120.60	265.40	3099 00	3477.50	1725.5	188.7	1914.4	5394 4	14 69
Relative %	0 3	2 2	4 9	56.6	64.0	32.4	3.6	36.0	100	91 79
On tolbutamide treatment (200 patients)										
Absolute	28.20	122 45	253.32	2947.83	3331.7	1620.25	122.9	1743 15	5095 0	14 08
Relative %	0.5	2 4	5.0	58.0	65.9	31.7	2.4	34 1	100	87 97
On insulin treatment (100 patients)										
Absolute	12.00	164.45	293.00	2927.55	3397.00	1435.00	164.00	1599.0	4996 0	14 2
Relative %	0.2	3.3	5.8	58.7	68.0	28.7	3.3	32 6	100	88 66

All blood pictures were counted by the same person.

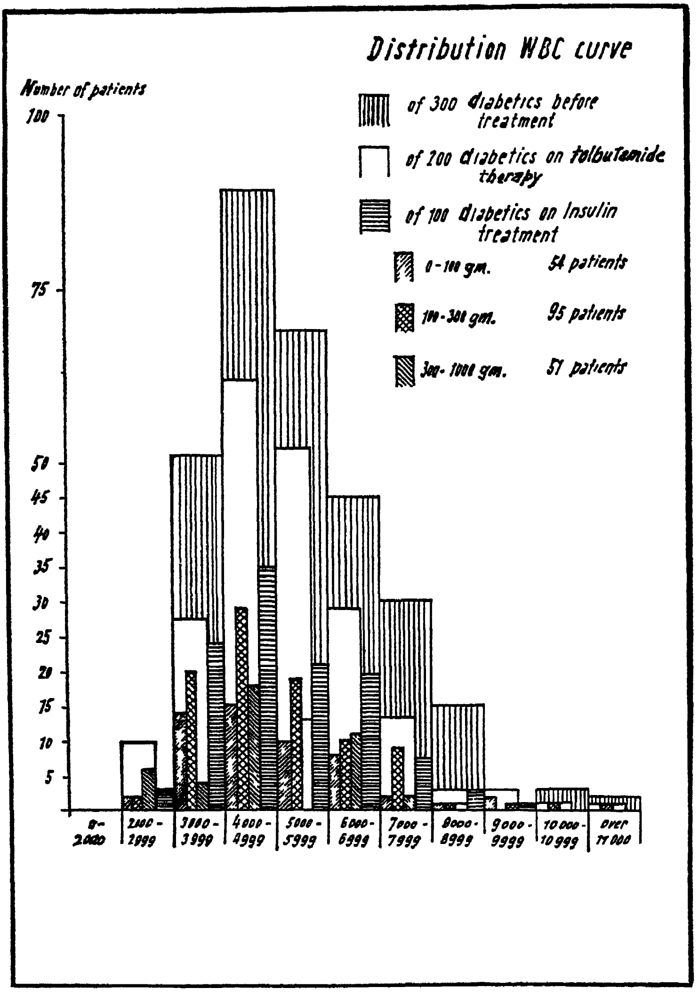

FIGURE 6. White blood cell distribution curve.

treatment. All these changes seem to us unimportant. When patients are arranged in groups that have received different total amounts of tolbutamide, the mathematical comparison shows that the leukocyte values in the groups with the highest doses of tolbutamide remain unchanged.

FIGURE 6 shows all patients, grouped according to their individual value of the WBC, subdivided into groups of 1000. The hatched columns illustrate the individual distribution of WBC of 300 patients before each treatment with tolbutamide or insulin. The white columns indicate the distribution of 200 patients on tolbutamide. The barred columns indicate the distribution of 100 patients on insulin. The white columns are subdivided into three groups with respect to the amount of tolbutamide administered. It can be

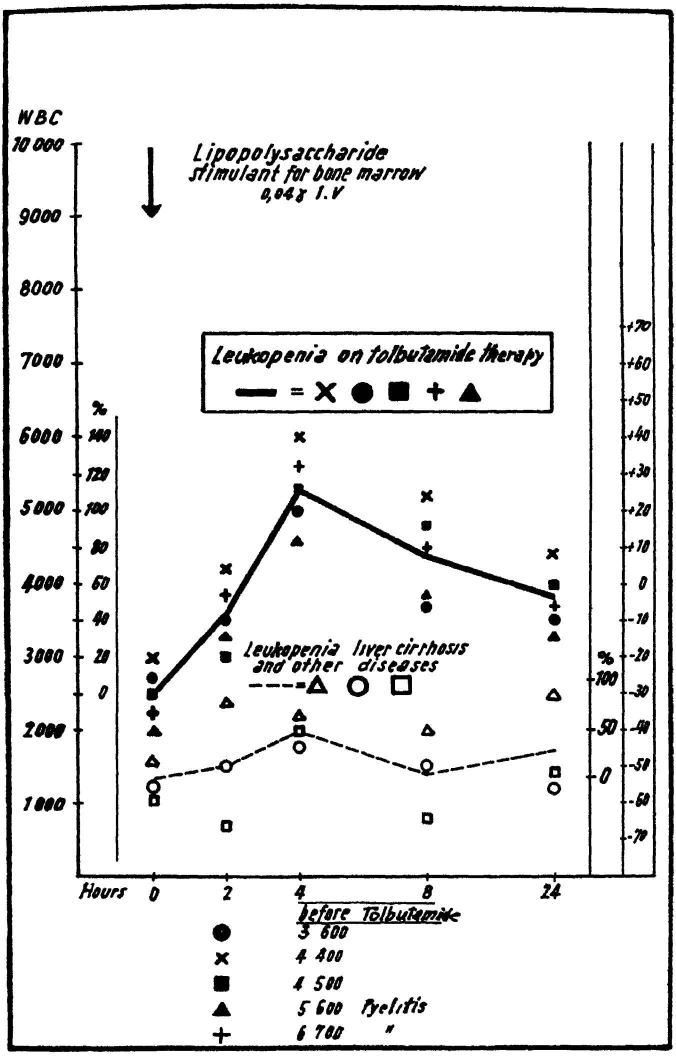

FIGURE 7. Five patients with leukopenia on tolbutamide therapy were tested for bone marrow function by administration of a lipopolysaccharide (0.04 γ I.V.) derived from *S. abortus equi* pyrexal (from the laboratory of Wander, Sigmaringen). The figures below the graph indicate the WBC count prior to treatment. The symbols in the graph show the individual values before the tests. The solid line shows the mean values for these 5 patients. The broken line gives the mean values for the comparison cases with leukopenia due to other causes; none of these patients were treated with tolbutamide.

seen that in all groups, with and without treatment with tolbutamide or insulin, the distribution remains the same.

Among the 200 patients treated with tolbutamide, leukopenia was evident in a total of 6 patients. The controls of the blood picture showed, for most of the patients, that this leukopenia was transient only, in spite of continued administration of tolbutamide. In further tests, a number of patients again had normal values; in some of these cases there had been an infection before.

Five of the patients showing leukopenia were tested intravenously with a lipopolysaccharide which is a bone marrow stimulant and can be used for functional bone marrow examination (FIGURE 7). This test showed, for the lowest values, an increase of the total numbers of leukocytes to twice the initial value after 4 hours. On the average, the increase was more than 100 per cent. Thus, functionally, the bone marrow is still able to pour out an ample amount of leukocytes. In contrast, we saw no increase of the total leukocytes or other blood components in cases of cirrhosis of the liver with leukopenia, or in cases of aplastic anemia, or in persons suffering from cancer and treated with cytostatics. Thus, in patients who show leukopenia during treatment with tolbutamide, the bone marrow is not exhausted. Since injection of these small amounts of lipopolysaccharide is tolerated by the patients without complaint, I think that this test may be recommended for obtaining information as to the reserves in the bone marrow. Body temperature did not rise more than 1° F., and no disturbance of the good general condition was observed.

Effects of Tolbutamide on Blood Sugar Level and on R. Q.

In FIGURE 8 we see the blood sugar curve in an extended glucose tolerance test. The dotted line indicates the control test, and the solid line the level

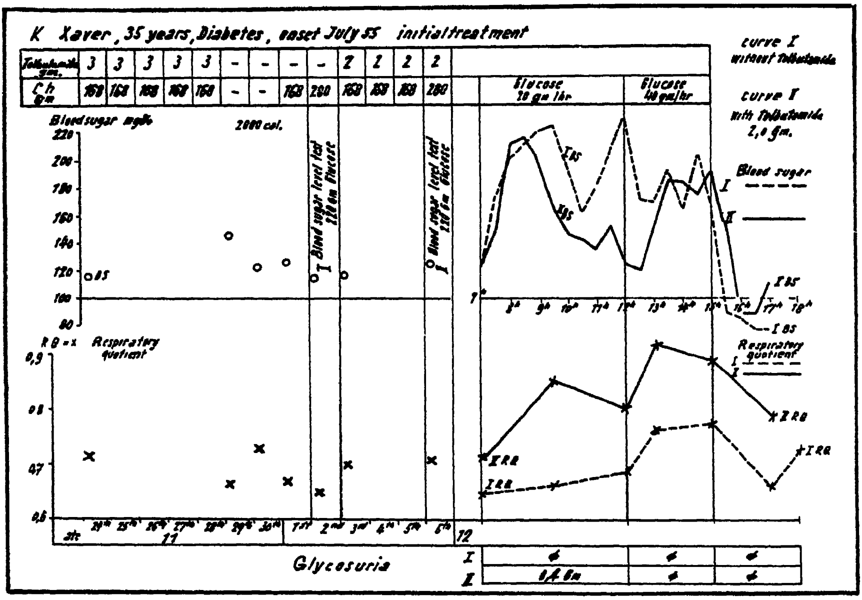

FIGURE 8. The upper part of the graph shows the total carbohydrate intake and the tolbutamide therapy, and then the fasting blood sugar and the blood sugar curve on the days of the extended carbohydrate tolerance tests. The lower part of the graph shows the values of the R. Q. during treatment and on the days with extended carbohydrate tolerance tests.

The solid curves show the blood sugar level and the R. Q. under tolbutamide treatment; the broken curves, without tolbutamide.

after administration of tolbutamide. Glucose was given orally, 20 gm./hr. for 5 hr. and 40 gm. per hr. for 3 hr.

The blood sugar curve under tolbutamide administration was more nearly normal. On the other hand, the R. Q. increases under tolbutamide to values greater than 0.9, whereas it reaches only 0.75 without treatment. This seems to be an indication that glucose utilization is increased, as it is after insulin. Thus, the increase in the R. Q. corresponds to the increased oxidation of sugar.

Thus, in particular cases under treatment with tolbutamide, subjected to extended administration of carbohydrates as a functional trial, a distinct increase of the R. Q. can be proved.

The Effect of Tolbutamide on the Blood Sugar Level of Normal Subjects after Intravenous Infusions of Glucose (25 gm.) over a Period of 1½ Hours

For the test, normal subjects received 3 gm. of tolbutamide on the day before and were subjected to 2 intravenous, continuous functional trials over 1½ hours on the day of the test, with a total of 25 gm. of glucose in each trial (FIGURE 9). The same persons served as controls. In comparison with

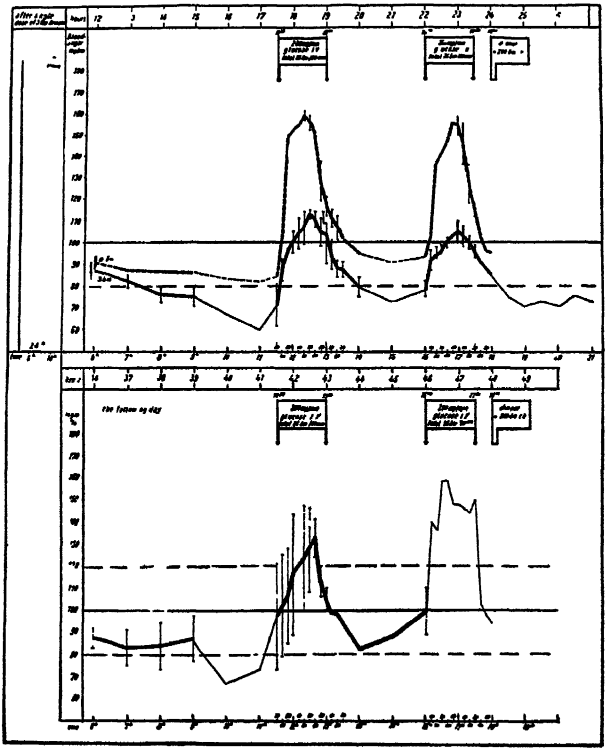

FIGURE 9. Blood sugar level test in 2 normal fasting subjects (male, age 54; male, age 38). The brackets show differences greater than 5 mg. Solid line, with tolbutamide; broken line, without tolbutamide.

the functional trials without previous administration of tolbutamide, the blood sugar level rises less and declines faster; that is, the glucose disappears speedily from the circulation. After the following supper (24 hours after the intake of tolbutamide at 6 P.M.), containing 200 gm. of carbohydrates, no new increase of the blood sugar occurred. Thus, the total sugar intake of these easily absorbed carbohydrates is intercepted by the liver, and the blood sugar level remains in the hypoglycemic range. We can observe the same behavior after an overdose of insulin; for instance, during psychiatric insulin shock treatment.

Discussion

Thus, it has been found that the action of tolbutamide in the healthy subject begins remarkably fast and proceeds physiologically in the same way as an intensified insulin action.

Further investigations concerning the inactivation and physiological neutralization of the excretory product were carried out at our clinic. In addition, the quantitative determination of both tolbutamide and its inactivation product were studied:

(1) Tolbutamide is inactivated by oxidation to a substituted benzoic acid, which is the main excretory product; so far, no coupling product with glycol has been found, in contrast to the excretion product of pure benzoic acid (hippuric acid).

(2) The volume of the urine seems, within very wide margins, *not* to be limiting for the excretion.

(3) The physiological neutralization of the excretory product with ammonia requires, in an extreme case, no more ammonia than that corresponding to the difference between the maximum and the minimum of normal daily excretion of ammonia.

(4) There is an intense color reaction on the basis of the diacetylmonoxime plus *N*-phenylanthranilic acid, for the physiologically active substance; the inactive product reacts with only one thirtieth of the color strength. Conversely, in iodometric acid titration, the inactive compound reacts about 10 times more strongly than the active compound.

Summary

During one and a half years of observation, treatment with tolbutamide has proved useful in 500 patients. It affords a great relief for the older diabetic. Thus, strict dietary treatment in combination with oral administration of blood sugar lowering substances is again of great importance. Tolbutamide has thus far proved completely nonpoisonous.

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CONCLUDING REMARKS

By Rachmiel Levine

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It is very difficult, of course, to attempt a summary of the material here presented or to reconcile some of the contradictory data obtained by various investigators. I shall therefore confine my remarks to major impressions from the presented material. There is first the practical aspect, as summarized in the clinical studies and in the observations of side effects and toxicities. It would appear from these that the sulfonylureas can and do control glycosuria and hypoglycemia in the adult-onset diabetic with a frequency of success varying from 60 to 80 per cent. It has been emphasized repeatedly that it is imperative that those treated be subject to the same dietary control as is employed for insulin therapy. It has also been shown that any complication, such as an infection, may necessitate the temporary use of insulin; therefore, instruction in the administration of the hormone must never be neglected. The side reactions and toxic effects encountered with carbutamide have led to its withdrawal from therapeutic use. Thus far, tolbutamide seems to show fewer reactions, all of them mild. It would seem that the average daily dose lies somewhere between 1 and 1½ gm., and it might be safe to set a maximum of 2 gm. per day.

Despite many unclear points it does seem that the sulfonlyureas act by eliciting insulin secretion in animals and in human beings in whom a sufficient number of β cells are still present. There seems also to be an action upon insulin turnover in liver and peripheral tissues, since potentiation of insulin action can be obtained at high dose levels in the absence of the pancreas. There is also evidence that the glucose production and/or output by the liver are inhibited by the drugs at some unspecified point. This has been shown clearly only with high doses of the drugs.

To me the most important aspect of the research in this field has been the stimulus it has provided to renewed work on the etiology of diabetes mellitus and on the synthesis of insulin, its storage, and the control of its release. We may say that, in addition to stimulating the β cells, the sulfonylureas have stimulated the investigators.

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DETERMINATION OF SR^{90} AND BA^{140} IN BONE, DAIRY PRODUCTS, VEGETATION, AND SOIL*

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Introduction

Fission product contamination in many natural materials requires an efficient chemical procedure for the separation, purification, and absolute measurement of certain isotopes at very low levels. As part of the study of the distribution of long-range fall-out from nuclear tests, a technique for the determination of both strontium-90 and barium-140 has been developed. The sample materials include bone, cheese, milk, vegetation, and soil. The first four sample types contain calcium phosphate as a major part of their ash. The separation of the phosphate from calcium, strontium, and barium is necessary for these sample types. Soils contain exchangeable calcium as well as nonexchangeable calcium in the silicate lattices. Since strontium and barium will follow calcium under the proper conditions, procedures were designed around the chemistry of calcium, which acts as a carrier for strontium and barium. In all cases the preliminary chemical procedure yields a calcium chloride solution carrying the radioactive strontium and barium. The radioactivity measurements are made on the daughters of strontium-90 and barium-140, that is, yttrium-90 and lanthanum-140, respectively, which are extracted from the solution.

Preliminary Chemical Procedures

Bone, cheese, milk, and vegetation. The main objective of this phase of the procedure is to obtain a pure solution containing the mother radio-element (Sr^{90} and Ba^{140}) which is in a form suitable for "milking," that is, the quantitative extraction of the daughter. The separation of the daughter (Y^{90}, La^{140}) from the mother (Sr^{90}, Ba^{140}) is rather difficult in solutions containing phosphate ion, since the phosphates of the two groups precipitate in a very narrow *p*H range. Alkaline earth phosphates precipitate at a *p*H of about 2.4, while rare earth phosphates come down at about 1.5. In this procedure *p*H control is difficult, since the solution is hot and the ammonia content varies with time and temperature. For these reasons it is desirable to separate the phosphate ion from the alkaline earths.

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Samples of all of these materials are first completely ashed at about 600° C. The ash containing the strontium and barium in calcium phosphate is then taken up in an excess of concentrated hydrochloric acid and the solution filtered to remove insolubles.

In order to separate the phosphate ion, ammonium hydroxide is added to the clear filtrate to bring the *p*H to 1.5, after which ammonium oxalate is added in excess. The calcium oxalate forms, carrying with it the strontium and barium quantitatively, leaving the phosphate ion in solution. The calcium oxalate is filtered and ignited to calcium oxide at 600° C. The completeness of removal of the phosphate is tested by dissolving the calcium oxide in hydrochloric acid and raising the *p*H above 3.0. Formation of a precipitate indicates that appreciable phosphate was carried down in the oxalate precipitation. If such a precipitate forms, the *p*H is lowered to 1.0 again (dissolving all of the phosphate) and the oxalate precipitation is repeated. Once the calcium chloride solution is found free of phosphate, the preparation of the mother solution is complete, and it may be milked repeatedly for Y^{90} and La^{140} without loss of Sr^{90} and Ba^{140}.

Soil. The exchangeable calcium, Sr^{90} and Ba^{140}, in soil may be removed by two leaching procedures:

(1) A 1000-gm. aliquot of the original sample of soil is mixed with 4 liters of a 1 N ammonium acetate solution and allowed to stand for 24 hr. The sample is then filtered and washed with 500 ml. of 1 N ammonium acetate and the filtrate evaporated to dryness. The residue is dissolved in 6 N HCl and the calcium is precipitated as the oxalate at a *p*H of 1 to 1.5.

(2) Two liters of 6 N HCl are added to a 1000-gm. aliquot of the soil sample and allowed to stand for 24 hr. The mixture is then filtered and washed with 250 cc. of 6 N HCl. The filtrate is evaporated to dry-

ness and ashed at 600° C. for 15 hr. The residue is dissolved in 6 N HCl and sufficient hydrogen peroxide is added to convert ferrous to ferric iron. The *p*H is then raised to 7.5 by the addition of NH_4OH and the hydrous ferric oxide filtered off. This solid is dissolved and reprecipitated under the same conditions previously described. The filtrates are then combined, and the calcium is precipitated as the oxalate.

The portion of the Sr^{90} or Ba^{140} in a form insoluble in NH_4Ac or HCl is obtained by fusing about 160 gm. of Na_2CO_3 with 40 gm. of soil, followed successively by solution in water and 3 N HCl. After the siliceous residue is filtered off, the alkaline earth is precipitated. No appreciable Sr^{90} activity remains in this siliceous residue, as may be proved by dissolving several samples of this siliceous residue completely and then carrying out radiometric assay.

In all cases the oxalate is reprecipitated and then ignited to the oxide and weighed in order to determine the calcium content. The oxide is then dissolved in dilute HCl, producing the mother solution.

Milking Procedure

The milking of the mother solution for the daughter isotopes is essentially a two-step process designed (1) to separate the yttrium and lanthanum daughters quantitatively from the alkaline earth mothers, and (2) to produce a precipitate of the daughters suitable for radioactivity measurement. Separation is accomplished simply by raising the *p*H of the mother solution to about [illegible] with ammonium hydroxide after adding about 10 mg. of nonradioactive yttrium carrier. A white gelatinous precipitate of yttrium hydroxide (carrying with it the yttrium-90, lanthanum-140, and the rare earths will quickly form and coagulate satisfactorily if heated for a few minutes This precipitate is filtered, the time of precipitation is recorded, and the filtrate is stored for later milkings when the daughters have again appeared. Other long-lived rare earth fission products and natural radioactive isotopes that are carried with the alkaline earth oxalate precipitation are separated in this first milking. A second milking of the mother solution is generally required to obtain only yttrium-90 or lanthanum-140.

The gelatinous nature of the hydroxide precipitate makes it unsatisfactory for counting. Therefore, it is dissolved off the filter paper with 6 N hydrochloric acid, the *p*H is adjusted to the point just below precipitation, and excess oxalic acid is added This precipitates yttrium oxalate, an ideal solid for measurement of radioactivity because of its coarse granularity. The precipitate is filtered on a stainless steel funnel with suction and dried by pulling air through the sample for about ten minutes. The filter paper with the sample is then removed, mounted for counting on a brass disk, covered with Pliofilm, and secured with a brass ring.

Yttrium carrier completely free of natural radioactivity is very difficult to obtain. After trying several sources, reasonably good yttrium oxide was obtained from A.D. MacKay Co., New York, N.Y. The oxide is purified further by dissolving it in HCl, precipitating it as the hydroxide, converting it to the oxalate, and igniting it to the oxide. Once the yttrium carrier is pure it may be reused repeatedly for successive milkings. Carrier used for first milkings is saved and repurified. It is convenient to use unselected C.P. Yttrium oxide from any source for first milkings of soil and vegetation samples, since they are normally discarded. On the other hand, the bone and milk samples are often so free from natural or artificial contamination that the first milking can give a reliable assay. In this case, of course, the nonradioactive carrier must be used.

Radioactive Procedure

The radiometric determination of the final precipitate is done in a specially designed beta counter[1] that utilizes anticoincidence shielding to obtain low background.

The relatively short half lives of the daughter products, 64.24±.30 hours for yttrium-90 and 40 hours for lanthanum-140, make it desirable to count these isotopes rather than the parent isotopes strontium-90 (19.9 years) and barium-140 (12.8 days) for two reasons: (1) the decay of the daughter isotopes may be followed to detect contamination; and (2) repeated milkings from the same mother may be made after only a few days.

In the case of samples several months old only Sr^{90} is detected. If the Ba^{140} and Sr^{90} are present in similar but high concentrations, they may be conveniently analyzed together, either by a detailed analysis of the mixed daughter decay curve or by milking a third time 2 weeks later. In the case of similar but very low level activities of both Sr^{90} and Ba^{140} it is desirable to pass the $CaCl_2$ solution through an ion exchange column after adding nonradioactive strontium and barium carriers to effect the separation of the Ba^{140} and Sr^{90} prior to milking. The apparatus consists of a 2.2 × 20 cm. column of 200 to 400 mesh Dowex-50 cation exchange resin (8 per cent cross linkage). The sample is placed on the column as the chloride, and the column is eluted with 4 N HCl at a rate of approximately 0.3 ml./min. Approximately 5 1/2 hours are required to complete a collection. Calibration was carried out with radioactive strontium, yttrium, barium, and lanthanum. Stable calcium was determined spectrometrically. Under the prescribed condition no traces of calcium (peak at 192 ml.), strontium (peak at 25^{6} ml.), and yttrium (peak at 288 ml.) remain at the onset of barium elution. Barium peaked at 672 ml. and therefore only two collections need be made: the first from the first drop of eluant to 480 ml., and the second from 481 ml. to 832 ml. After this separation the two solutions are milked as described above.

Results

Over one hundred samples have been run by this method. To evaluate the absolute accuracy of this procedure the several steps will be discussed separately.

Recovery of calcium. The completeness of the conversion of calcium phosphate to calcium oxalate was defined by gravimetric analysis of pure calcium phosphate samples. It was found that more than 98 per cent of the calcium originally present as phosphate is recovered as calcium oxalate.

Recovery of strontium. A radioactive spike of strontium-90 was added to a solution of "dead" calcium phosphate, which was precipitated as phosphate and filtered. This precipitate was then taken through the procedure described above. The results showed that at least 98 per cent of the carrier-free strontium was recovered. Repeat milkings showed no evidence of measurable loss of strontium from the mother solution.

Efficiency of separation in milking. A series of samples was prepared using spikes of radioactive strontium. These samples gave count rates between 800 and 1300 counts per minute. The half life of yttrium-90 was derived by observing the decay of the mounted samples.[2] Counting the samples after more than six half lives of yttrium-90 have elapsed indicated that the separation of yttrium-90 from strontium-90 in the milking is quantitative. TABLE 1 lists the data for this experiment.

Reproducibility. Many of the samples have been milked two or more times. TABLE 2 lists the data on several of these natural samples, indicating the degree of precision obtained by this method.

Soil extraction. It was necessary at the outset of the program to establish the reproducibility of the extraction of "available calcium and Sr^{90}." TABLE 3 shows that aliquots of the same soil give an average deviation of approximately 1 per cent on duplicate runs.

Although the leaching procedure is fairly reproducible, one ammonium acetate treatment does not remove all of the Sr^{90} that is present (TABLE 4). Several samples from the New York, N.Y., area were leached two or three times. Each set showed a successive decrease of the Sr^{90}/Ca ratio, indicating that the Sr^{90} is more readily available than the calcium, and that at least 80 per cent comes out in the first leach.

TABLE 1

EFFICIENCY OF SEPARATION OF SR^{90} AND Y^{90} IN MILKING

Sample No.	Initial cpm (Immediately after milking)	Final cpm (After more than 6 half lives)
HC-97	1087±15	0.03
HC-99	1171±20	0.03
HC-100	940±10	0.01
HC-101	1010±10	0.02
HP-100	1220±10	0.03
HP-101	860±9	0.02
HP-102	1000±10	0.01
HP-103	1260±11	0.01

TABLE

PRECISION DETERMINED BY REPEATED MILKINGS

Sample No.	Milk No.	Sr^{90} content (arbitrary units)
B-3	2	1.43±0.08
	3	1.44±0.08
B-16	1	0.20±0.04
	2	0.15±0.05
C-6	2	0.97±0.0
	3	1.14±0.05
C-10		0.85±0.09
	[illegible]	0.7[illegible]

A sample of soil from the same vicinity as that used for the NH_4Ac treatment was leached with 6 N HCl. A single treatment removed 99 per cent of the leachable activity, and the second and third leaches removed calcium in successively smaller quantities (TABLE 4). Finally, the leached soil was fused with Na_2CO_3 to determine the Sr^{90} in nonexchangeable form. Apparently this contributes about 40 per cent of the total in this sample.

It is concluded that the experimental procedures for low level measurement of Sr^{90} and Ba^{140} in this variety of materials give satisfactory results.

TABLE 3

REPRODUCIBILITY OF CALCIUM EXTRACTIONS
(NH$_4$ AC METHOD)

Sample No.	*Sample size (grams)*	*Ca (gm.) extracted*
17A	1000	0.0612
A′	1000	0.0606
16A	1000	1.952
A′	1000	2.020
37A	1000	0.0936
A′	1000	0.0912

TABLE 4

EFFECT OF SUCCESSIVE NH$_4$AC AND HCl LEACHES OF SOIL SAMPLES FROM PALISADES, N. Y.

Sample No.	*Method*	*Leach*	*Ca (gm.)*	*Cpm*	*Cpm/Ca*
S-1	NH$_4$Ac	1st	3.97	35.5±.2	8.94
S-1		2nd	1.69	6.21±.02	3.67
S-1		3rd	0.55	1.32±.18	2.40
S-4	HCl	1st	2.06	164 ± 6	—
S-4		2nd	0.89	≤.8	—
S-4		3rd	0.45	≤.6	—
S-4		fusion	—	110 ± 10	—

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TRANSPLANTATION OF FINS IN XIPHOPHORIN FISHES*

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Introduction

Held (1951) successfully interchanged the dorsal and anal fins of the platyfish *Xiphophorus maculatus*. He found that, soon after the transplantation from their normal position to another site, the fins sloughed off down to their bases; then, about four weeks later, some of the fins began to regenerate from their intact basal stumps. Regardless of their atypical position, the autotransplanted anal fins regenerated into anal fins and the transplanted dorsal fins regenerated into dorsal fins. If anal fin transplants made in females were exposed to androgenic substances, the reforming anal fins attained some features characteristic of the gonopodia of males, but the regenerating dorsal fins were not influenced by androgens. When Held attempted to transplant fins from one platyfish to another of its siblings, all the homotransplants failed. He attributed the failure to genetic differences between the donor and host in his platyfish stocks.

The Genetics Laboratory of the New York Zoological Society had a highly inbred stock of platyfish that had been developed by brother-sister matings for seventeen generations, actually since 1939. It also had other platyfish that had been inbred for fewer generations and still other fish that were not inbred at all. These stocks of platyfish seemed suitable for evaluating the role of genetic factors in the success or failure of fin transplantation. We anticipated that transplantations of fins from one platyfish to its siblings would succeed in the members of our most highly inbred stock and, perhaps, in some of the others. This is the report of our experiments.

Material and Methods

Terminology. An autotransplant denotes a transfer of tissues from their original site to another part of the body of the same individual.

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An isotransplant represents a tissue transplant between a host and a donor that are members of the same highly inbred strain.

The term homotransplant refers to tissue transferred from one individual to another member of the same species, but not to members of the same highly inbred strain.

A heterotransplant involves a transfer of tissues between members representing different species or different species hybrids.

A successful transplant is one that is maintained in a living state indefinitely. A successful fin transplant may undergo initial and partial degeneration (sloughing), after which it regenerates and subsequently survives. Indications for success of a fin transplant could be noted, in our experiments, six to eight weeks after the transfer. Those that were intact and alive for eight weeks survived as long as the host animal. Some remained alive for as long as two years.

Soon after a fin has been transplanted, the distal portion usually begins to degenerate, but this degeneration, or sloughing, may stop at various levels. Eventually, regeneration of the transplanted fin stems from the base of the intact fin.

Genetic history of fishes. Various genetic stocks of *X. maculatus* (TABLE 1), which differed with regard to the degree of inbreeding, were selected.

TABLE 1

SUMMARY OF TRANSPLANTATION EXPERIMENTS

Pedigree		*Inbred*	*Type of*	*Fate*		
Host	*Donor*	*generations*	*transplant*	*Failures*	*Successes*	*Total*
30	30	17	Iso	0	14	14
163	163	6	Iso	2	11	13
Bh, gray*	Bh, gray*		Homo	3	1	4
Bh, red*	Bh, red*		Homo	4	0	4
492	492		Homo	4	1	5
500**	500**		Homo	2	9	11
501	500**		Homo	1	3	4
427	427		Homo	14	0	14
274	274		Homo	39	1	40
374	374		Homo	6	2	8
457	457		Homo	6	2	8

*Inbred for seven generations, but not by brother-sister matings.

**F_1 offspring of $Bh^7 \times 30^{16}$ (the 30^{16} parent inbred for 16 generations).

Strains 30^{17} and 163^6 originally came from the Rio Jamapa population. The former had been inbred by brother-sister matings for 17 generations; the latter, for only 6 generations.

The spotted platyfish of the Bh^7 strain were inbred in part for 7 generations, but not by brother-sister matings. One substrain was red, the other gray. Strain 492 was produced by mating members representing the two Bh^7 strains.

Fish of pedigree 500 were the F_1 offspring of crosses between red Bh^7 and 30^{16}; those of 501 were the F_1 offspring from matings of gray Bh^7 and 30^{16}. The fish of strains 274, 374, 427, and 457 were not inbred.

Technique. All stock fish were kept in conditioned aquarium water according to the methods described by Gordon (1950). Prior to the operation, the donor and the host were anesthetized in a dilute solution of Tricain Methanesulfonate (Sandoz's MS 222). After the fish had received their fin grafts they were placed in water containing 0.67 per cent household salt to inhibit infection. The technique for the removal of the fins and their implantation was similar to that described by Held.

The donor fish was placed in a dish and held by wet cotton pads in such a way that the fin to be excised was uppermost. Working under the dissecting microscope, all scales around the base of the dorsal or anal fin were removed with a pair of fine forceps. Then a longitudinal incision, approximately 0.5 mm. on either side of the first fin ray, was made with a fine dissecting scalpel. The scalpel was inserted at an oblique angle so that the blade was pushed underneath the fin until it reached the distal segments of the radials of the dorsal or anal fin (according to Rubin and Gordon, 1953, the radials of the anal fin suspensorium in adult females and immature fish consist of three segments: the proximal spinelike *actinosts*, the median *mesonosts*, and the distal *baseosts*; the baseosts articulate with the fin rays). The cut was made in a posterior direction along the whole basal length of one side of the dorsal or anal fin, severing all principal muscles; the same procedure was then repeated on the other side of the fin. The fin was then grasped near its anterior end with a pair of curved forceps, and the radials of the fin were gently freed. The whole fin was immediately placed into "amphibian" Ringer's solution and all loosely adhering tissue fragments were removed from it. When the dorsal fin was obtained from an animal larger than the host, the anterior and posterior end of the fin were trimmed to a suitable size. Trimming the transplanted fin did not reduce its chances for successful growth.

The host was placed in a dish, and its exposed head and gills were covered with wet cotton. During the operation the experimenter's left index finger pressed gently on the wet cotton to hold the fish in place. The scales were removed from the transplantation site; then a small dorso-ventral incision was made. The scalpel was withdrawn and then reinserted to open the incision to about three quarters of the length of the base of the excised fin, and to a depth determined by the length of the fin's radial elements. With a pair of forceps the fin was held in such a way that its radials pointed toward the prepared pocket. The mouth of the pocket was forced open by a gentle downward motion of the forceps; then, without releasing the pressure upon the pair of forceps, the basal portion of the slightly compressed fin was fitted into the pocket. After the operation the fish was placed in an aquarium in semidarkness where it was left undisturbed for at least forty-eight hours.

In fish only a few days old a different method for the removal and implantation of the dorsal and anal fins was used. A small donor fish was placed on its side on a wet glass plate. The dorsal or anal fin was removed with a razor blade by cutting slowly in an anterior-posterior direction about one half of a millimeter below the fin. The severed fin was immediately placed into Ringer's solution and the loose adhering tissue was removed with two sharp needles. A host fish was placed on smooth wet filter paper. With a fine glass needle a dorsoventral slit was made in the myotomes; the loose scales around the opening were removed. The base of the excised fin was gently pushed into the prepared slit. The very young host fish were kept in a glass container containing 500 cc. of Holtfreter's solution.

The best site for fin implantation was found to be in either side of the caudal peduncle just posterior to the anal fin. There, the myotomes are well developed and the incisions were sufficiently deep to receive the grafts neatly (FIGURE 1). In larger fish the operation was easier.

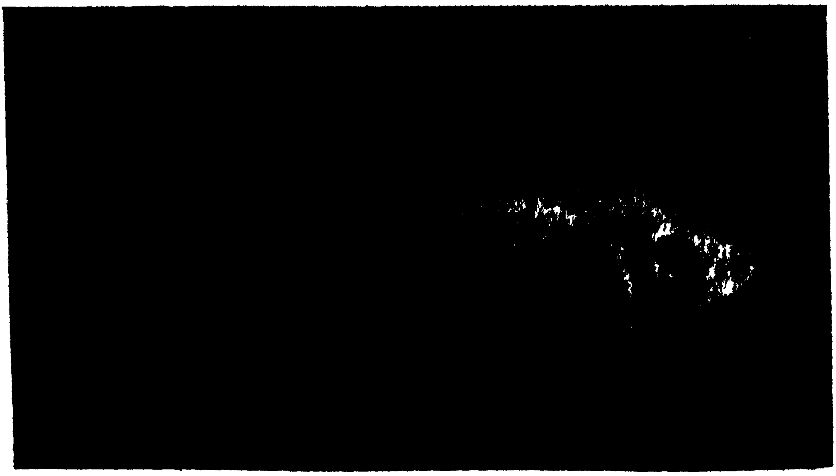

FIGURE 1. A live female platyfish of strain 30^{17} showing a successful isotransplant of an anal fin in the musculature of the caudal peduncle near the lateral line. Photo by Sam C. Dunton.

Histological examinations were made of normal fins, of those successfully transplanted, and of those in various stages of degeneration. Fish were fixed in either Bouin's picroformol or formalin and were sectioned at 5 μ; the sections were stained with Harris's hematoxylin and eosin or with Masson's trichrome.

Results

Isotransplants. Six females and 2 males of the highly inbred strain 30^{17} received dorsal fins from male donor fish; 6 additional adult females received anal fin grafts from immature females (TABLE 2, Nos. 1 to 14). Between the third and sixth day after the operation, hemorrhagic areas appeared in nearly all grafts. After the eighth or ninth days, in each of 12 host fish the distal margin of the fin disintegrated, and then the membranous tissue between the fin rays sloughed away down to the base of the fin. Histological examination revealed that many large phagocytes had accumulated around the base of the fin rays and had engulfed the cellular debris; elsewhere, free erythrocytes were present in large numbers (FIGURE 2).

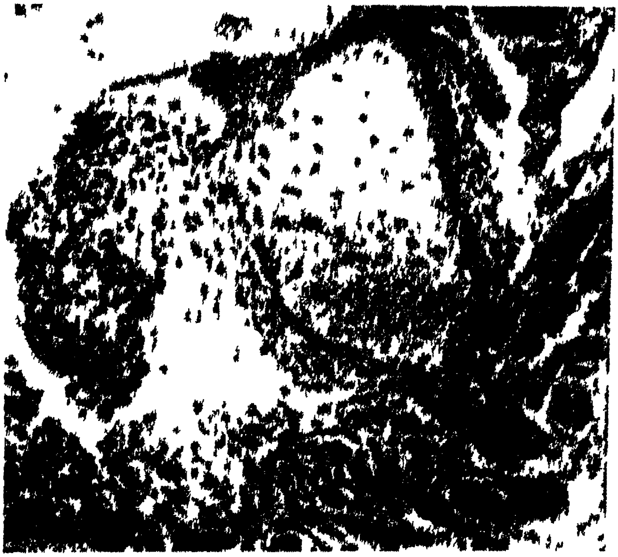

FIGURE 2. Cross section through an isotransplant of a dorsal fin in a platyfish of strain 30^{17} fixed and sectioned eight days after transplantation. Many phagocytic cells have accumulated in a band between a fin ray of the transplant and host myotomes. Very little cellular destruction or disorganization is apparent. Hematoxylin and eosin. ×215.

TABLE 2

HISTORIES OF TRANSPLANTED FINS IN ADULT PLATYFISH, *XIPHOPHORUS MACULATUS*

Pedigree	*Number*	*Sex*	*Fin*	*Sloughing in days*[*]	*Regeneration in days*[**]	*Survival in days*[†]
30[17]	1 to 6	♀	Dorsal	8, 9	28	S
"	7	♂	Dorsal	none	—	S
"	8	♂	Dorsal	9	26	S
"	9 to 11	♀	Anal	8, 9	21 to 26	S
"	12, 13	♀	Anal	8, 9	21 to 26	S
"	14	♀	Anal	none	—	S
500	15, 16	♀	Anal	4, 5	none	12 to 14
"	17 to 20	♀	Dorsal	3 to 9	22	S
"	21, 22	♀	Dorsal	5	22	S
"	23 to 25	♀	Anal	4 to 7	28	S
501	26	♂	Anal	4	none	50
"	27 to 29	♂	Anal	4	21	S
Bh, red	30 to 32	♀	Dorsal	3, 7	none	11 to 13
"	33	♀	Dorsal	5	none	63
Bh, gray	34 to 36	♀	Dorsal	17 to 19	none	22
"	37	♀	Dorsal	none	—	S
492	38 to 41	♀	Dorsal	3 to 7	none	12 to 14
"	42	♀	Dorsal	6	24	S
163[6]	43, 44	♀	Dorsal	3 to 5	none	9
"	45 to 48	♂	Dorsal	3 to 4	25	S
"	49 to 55	♀	Dorsal	3 to 4	25 to 30	S
427	56 to 69	♀, ♂	Both	3 to 7	none	7 to 9
274	70 to 91	♀	Both	3 to 9	none	5 to 14
374	92 to 97	♀	Both	3 to 8	none	4 to 14
457	98 to 103	♀	Dorsal	8, 9	none	8 to 13
"	104 to 105	♀	Dorsal	6, 7	25, 28	S
"	104[‡]	♀	Dorsal	3	none	6
"	105[‡]	♀	Dorsal	6	none	67
374	106, 107	♀	Dorsal	6, 7	25	S
274	108	♀	Anal	none	—	S
"	108[‡]	♀	Dorsal	8	none	62
"	109 to 125	♀, ♂	Both	?	none	8 to 11

[*] The numbers indicate the interval, in days, between transplantation and the onset of the sloughing process.

[**] The numbers indicate the interval, in days, between transplantation and the beginning of the regeneration process.

[†] S indicates successful fin transplants, which survived as long as their hosts. The numbers indicate in days the period before the disintegration of the transplanted fin.

[‡] Fish that received a second fin transplant.

In 2 of the 14 transplants no sloughing of fins took place. In the others the grafts appeared to be completely lost except for slight elevations at the points of their prior insertions. Three to 4 weeks later blastemas formed at the mouths of the pockets and, 4 weeks later, all grafts had regenerated. In 5 hosts, the regenerated fins were slightly deformed. This is not surprising because, in some, only parts of fins were transplanted and, in one, the fin graft was apparently inserted imperfectly. Histological study revealed that all fin grafts were well vascularized 3 months after they had been transplanted (FIGURE 3).

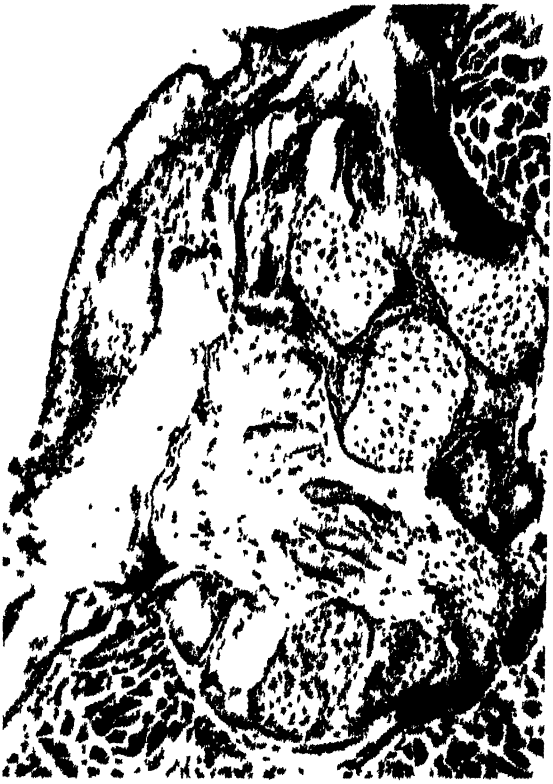

FIGURE 3. Cross section through the site of a successful isotransplant of a dorsal fin in a platyfish (strain 30^{17}) fixed three months after transplantation time. The basal rays of the transplanted fin are well imbedded and integrated in the myotomes of the host. Hematoxylin and eosin. ×100.

The dorsal fins of 6 donors used in the above transplantation experiments possessed erythrophores. When these 6 transplanted fins regenerated, after first having sloughed off, their erythrophores reappeared (TABLE 3, Nos. 1 to 6).

TABLE 3

FATE OF THE PIGMENT CELLS IN THE TRANSPLANTED FINS

Fish number	*Pigmentary genes* of*		*Pigment cells present in transplanted fin*	
	Host	*Donor*	*Originally*	*Three months after*
1 to 6	dr	dr	Erythrophores	Erythrophores
7 to 14	dr	+	None	None
27 to 29	+	r	Erythrophores	Erythrophores
106	+	dr	Erythrophores	Erythrophores
45 to 47	sd dr	+	None	None
51 to 55	sd dr	+	None	None
50	sd	+	None	None
48, 49	+	sd dr	Erythrophores, Macromelanophores	Erythorphores, Macromelanophores

**Genes*	*Names*	*Chromatophores*	*Position*
sd	Spotted-dorsal	Macromelanophores	Dorsal fin
dr	Dorsal-red	Erythrophores	Dorsal fin
r	Red	Erythrophores	Over-all
+	Multiple recessive	Micromelanophores only	Over-all

All fishes had micromelanophores in addition to their other chromatophores.

Homotransplants. (1) In the platyfish of the inbred strain 163, 11 of 13 transplantations of dorsal fins were successful (TABLES 1, 2). Two dorsal fins containing macromelanophores and erythrophores were successfully grafted into the caudal peduncles of 2 hosts that did not possess either of these chromatophores in their dorsal fins. Both types of pigment cells appeared in the 2 fins that regenerated (TABLE 3, Nos. 48, 49). In the reverse experiment dorsal fins that were free of these pigment cells were transplanted to hosts that had pigmented dorsal fins. Neither macromelanophores nor erythrophores appeared in the regenerated fins (TABLE 3, Nos. 45 to 47, 51 to 55).

(2) Each of the 4 fin transplants made between red members of the Bh^7 strain (TABLE 2, Nos. 30 to 33) was negative. One of the 4 transplants between members of the gray Bh^7 strain was successful. It did not pass through the characteristic sloughing process (TABLE 2, Nos. 34 to 37). Also, only 1 graft, out of 5, was successful between members of strain 492 (TABLE 2, Nos. 38 to 42).

(3) The 11 dorsal or anal fin transplantations that were made between members of pedigree 500 (TABLE 2, Nos. 15 to 25) first sloughed off but, after 3 to 4 weeks, 9 of the 11 regenerated.

(4) Four orange-yellow anal fins pigmented by xanthoerythrophores were removed from 4 females of pedigree 500 and were transplanted into the caudal peduncles of 4 sexually mature males of pedigree 501 (TABLE

2, Nos. 26 to 29). After the ninth day, the anal fin grafts in all 4 hosts had sloughed off, leaving only tiny spots of orange tissue. After 3 weeks, 3 anal fins began to regenerate and redevelop xanthoerythrophores (TABLE 3, Nos. 27 to 29). The fourth graft persisted for 7 weeks as a tiny speck of orange-colored tissue.

(5) Fifty-three transplants were made with sexually mature fish of strains 274, 374, 427, and 457 (TABLE 2, Nos. 56 to 108). Only 5 transplants were successful (TABLE 2, Nos. 104 to 108); in all but 1 of these (No. 108) the fins passed through the characteristic sloughing process, then regenerated after 3½ weeks. One of the transplanted dorsal fins that possessed erythrophores (TABLE 3, No. 106) regenerated a new dorsal fin that developed erythrophores. Three months after the first transplantation, each of these 5 fish received a second graft from a donor unrelated to the primary donor. Fish 105, 107, and 108 received dorsal fins removed from members of strain 274. Fish 104 and 106 were implanted with fins from members of strain 500. The implants in 106 and 107 became detached; the one in 104 sloughed off rapidly and disappeared completely on the sixth day. In fish 105 and 108, the lower third of the graft withstood the sloughing process for 2 months; then the secondary grafts finally disintegrated, but the primary fin grafts in all 5 fish remained unchanged (FIGURE 4).

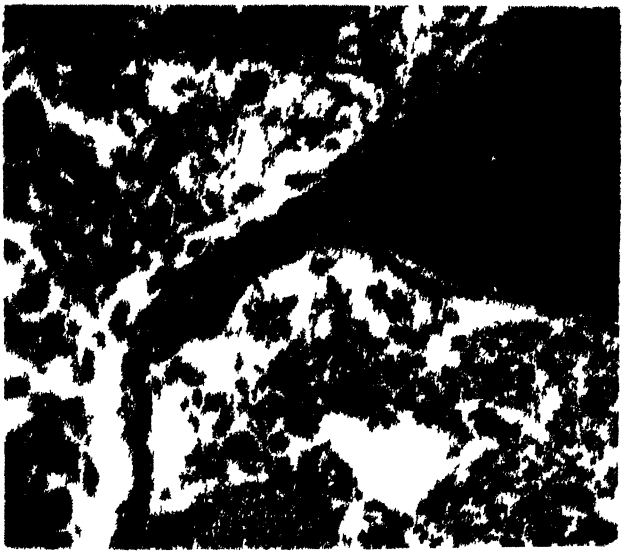

FIGURE 4. Cross section through a homotransplant of a dorsal fin in a platyfish (strain 427) eight days after transplantation. This transplant differs from the transplant in FIGURE 3 because large numbers of erythrocytes are scattered freely throughout the tissue spaces. The tissues surrounding the degenerating fin ray have been destroyed. Hematoxylin and eosin. ×215.

(6) In order to determine whether the age of the host influences the success of fin transplantation, 17 grafts were made between siblings of strain 274 which were less than 2 days old (TABLE 2, Nos. 109 to 125) (it is well to remember that platyfish are born about 21 days after fertilization of the eggs, according to Hopper (1943), so that a 2-day-old platyfish is actually in its twenty-third day of life). All grafts were found intact after 3 days but, after the eighth day, only traces of 11 grafts were seen. After 11 days none were left.

Discussion

Approximately one third of Held's autotransplants of dorsal and anal fins of adult *Xiphophorus maculatus* were successful, but all his homotransplants failed. In his opinion the failure of his homotransplants was due to the heterozygosity of the host and donor fish. Earlier, Goodrich and Nichols (1933) obtained somewhat comparable results when they attempted to transplant scales and their surrounding tissues in the goldfish. Their autotransplants were successful, but their homotransplants disintegrated. Other attempts at homotransplantation of various tissues in fish by Secerov (1912), Sauter (1934), and Nardi (1935) were failures.

In transplanting fins from one individual to another we used highly inbred strains of fish for the first time and were successful. Our results correspond to those of others who used highly inbred animals such as rats and mice. Close genetic correspondence between host and donor in adult *X. maculatus* is definitely of prime importance in the successful transplantation of fins from one animal to another. Fin transplantations between the members of our highly homozygous strain 30^{17} were most successful. In transplants between members of strain 163, which had been inbred for fewer generations, the results were slightly less successful. The chances for successful homotransplants are enhanced when host and donor have at least one similar set of chromosomes; this was the case in our strains 500 and 501 (TABLE 1). In homotransplants between members of our heterozygous strains the number of successful grafts was small.

No sloughing occurred in only two isotransplanted and two homotransplanted fins. But usually, after a fin had been transplanted—and this applies to our iso- and homotransplants and Held's autotransplants—the sloughing process set in. In sloughing, after the transplanted fin had regained its vascularity, the blood vessels became distended, the blood flow stopped, the vessels ruptured, and blood entered the intercellular spaces. Since sloughing of fins occurred in all categories of fin transplants, it is unlikely that the degenerative process can be attributed to tissue antagonism. We are not prepared to compare the sloughing process in fin grafts to a somewhat similar process in the degeneration of skin grafts in mammals.

Within the limited scope of our experiments the age of the post-embryonic host did not affect the outcome of our transplantations. Recently Humm and Young (1956) reported some success in transplanting fragments of ectoderm, neural tube, and mesoderm of platyfish-swordtail hybrid embryos into pure swordtail host embryos.

The pigment cell patterns in the transplanted fin may disappear during the sloughing process, but they reappear in the regenerated fin essentially the same as before and are in no way affected by the pigmentary genes of the host (TABLE 3). In their experiments in transplanting scales in the goldfish, Goodrich and Nichols (1933) found that the pigment cells in the tissues surrounding the scales retained their identity in their new sites. Marcus and Gordon (1954) showed that transplanted melanoma cells in xiphophorin fishes recreated a state of melanosis in their new site. The size, shape, and polarity of transplanted fin rays and scales were maintained in fishes in the experiments that have been described by Birnie (1947) and Nardi (1935).

Summary

(1) Homo- and isotransplantations of anal and dorsal fins were made between members of various strains of platyfish, *Xiphophorus maculatus*, in order to study the role of genetic factors in transplantation.

(2) Nearly all transplanted fins sloughed off three to nine days after the operation. The degeneration began at the distal margin of the fin and progressed toward the base, stopping at various levels. Eventually a new fin regenerated, if the base of the transplanted fin remained intact.

(3) The results from the transplantation of anal and dorsal fins between members of highly inbred platyfish were extremely successful. Those from transplantation of fins between members of less highly inbred platyfish were less successful. Results from transplantation of fins between nonrelated platyfish were quite poor.

(4) The pigment cells of successfully transplanted and regenerated fins redeveloped in accordance with the donor's genetic constitution for fin coloration.

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